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A CYTOLOGICAL STUDY OF HETEROTHALLISM IN FLAX RUST [1]

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INTRODUCTION

Blakeslee (*10*)[3] in 1904 announced the discovery that in certain mucors two parents that are alike in form but unlike in sex are necessary for sexual reproduction. This condition he called heterothallism. Since then heterothallism has been proved to exist in some species of all the main groups of fungi.

Present-day knowledge of heterothallism in the rusts began with the work of Craigie (*17*) in 1927. Information up to the present time (1934) is limited to species of *Puccinia* (*3, 4, 5, 17, 18, 19, 20, 21, 22, 30, 43*), *Uromyces* (*6*), and *Gymnosporangium* (*19*). Experimentally proved homothallism is reported in one species, *Puccinia malvacearum* Mont., by Ashworth (*7*). Outside of this small group of related genera, the subject of heterothallism in rusts is a broad unexplored field.

Within a single rust species there may be variants known as "physiologic forms" which differ from each other in virulence, in range of power of infection, and in geographic distribution. If such a species is also heterothallic, hybrids may form between its physiologic forms, with the result that new physiologic forms of this rust are generated, some of which may be a serious menace to the host. Work along this line is in progress in several laboratories (*34, 36, 40, 41, 50, 52*).

Flax rust (*Melampsora lini* (Pers.) Lév.), a member of the Melampsoraceae, represents a family of rusts in which heterothallism is unknown.

Melampsora has been studied from other points of view, however. Fromme (*29*), in 1912, made a detailed cytological study of *M. lini*, and Sappin-Trouffy (*44*), in 1896, Blackman and Fraser (*8*), in 1906, and Kursanov (*35*), in 1922, published brief studies of the aecium of several species of this genus. Lindfors (*37*), in 1924, studied nuclear divisions in *Melampsora*. Moss (*38, 39*), in 1928 and 1929, published cytological studies of the development of the uredium in several

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[3] Reference is made by number (italic) to Literature Cited, p. 789.

species of *Melampsora* and related genera. Tobler (*51*), in 1921, described the histological relations of flax to its rust. Buchheim (*14*), in 1915, by cross-inoculation of the rust on 12 species of *Linum*, learned that there are several physiologic forms of *M. lini*. Added data on this subject have been contributed by Pethybridge, Lafferty, and Rhynehart (*42*), Hart (*31*), and others. Hart (*31*) also contributed studies of physiological factors affecting germination of the spores and the growth of infections. Henry (*33*), in 1930, made crosses between immune and susceptible varieties of flax and made selections which combined immunity from rust with other desirable characters. Henry (*32*) also published an account of the rust in which control measures were recommended.

MATERIAL AND METHODS

The rusted flax straw bearing telia was received in the fall and kept through the winter. The material was divided and part was kept in a cool dry spot in the potting house, part out of doors on the ground in a partly shaded spot, and part on a box of damp soil on the balcony.

In inoculating, the rusted straw was sprayed with rain water from an atomizer; then a Petri dish was filled with mud and the bits of rusted straw were pressed into it with the rust exposed and then sprayed again. A glass tube about 4 inches in diameter was lined with wet paper toweling and set down over the seedlings, and the plants were sprayed with the atomizer until dewy. The Petri dish was inverted and set on the top of the tube, as a lid, with the rusted straw directly over the seedlings. Wet paper toweling was folded down over the top, a layer of cellophane was placed over that, and both were held in place with a rubber band. The plants were well watered and set under a greenhouse bench for 48 hours, then freed and placed on the bench. A light cage covered with tarlatan was set over the plants to prevent access of insects. Inoculations were made every other day throughout the season, so that after the work was well begun infections of all ages were available at any time. Greenhouse notes were kept of the cultures.

Material of all ages was fixed in Flemming's solutions of various strengths and in chromo-acetic-urea. Flemming's weak solution proved best for young flax leaves, while the solution of medium strength gave good results with older materials. The fixed material was washed and dehydrated and then embedded in paraffin. Sections were cut 10μ thick. The stains used were safranine and methylene blue for the first part of the work and iron haematoxylin counterstained with Congo red for the work on spermatia.

EXPERIMENTAL DATA

LIFE CYCLE

Melampsora lini, an autoecious, long-cycle rust, bears its spermogonia, aecia, uredia, and telia upon flax. Ordinarily, the life cycle follows the usual sequence. The teliospores germinate in the spring (April to June), and the promycelia bear sporidia. The sporidia grow into mycelia bearing first the spermogonia and later, if fertilization takes place, the aecia. Under the conditions of these experiments

the great majority of the infections are found upon the leaves of the flax plant. The few infections that appear on the stems are, however, larger and longer lived on an average than those upon leaves. The aeciospores grow into mycelia that bear urediospores. If these infections are upon leaves, and the great majority of them are so located, the urediospore-bearing period is usually prolonged until the leaf weakens and dies. Exceptionally, uredia on a leaf bear also a few teliospores. If, however, the infections are on stems, urediospore production occurs for a short time and is then superseded by teliospore formation. The teliospores form a solid, smooth, black crust that spreads up and down the stem for weeks or even months until the stem yellows and dies. Teliosporic crusts become 3 and even 4 inches long.

The uredial generation can be repeated. By transferring urediospores every 2 or 3 weeks to fresh young flax plants, a continuous series of uredial generations has been maintained for nearly 2 years without loss of vigor. The behavior is the same now as at the beginning of the series; the leaf infections bear urediospores in abundance and the stem infections run over quickly into teliospore formation.

The teliosporic generation does not repeat, and the teliospores require a rest period before germination. The limits of this rest period are not accurately known. Hart (*31*) found that teliospores of flax rust stored out of doors through the winter in Minnesota did not begin to germinate until early June, and all attempts to shorten or break the rest period by exposing the spores to chemicals or to variations of temperature and moisture were unsuccessful. In the mild Berkeley (Calif.) winter climate, however, spores can germinate after a much shorter rest period. Under these conditions, apparently, no extremes of temperature or of moisture are required for the "after-ripening" process. Plants grown out of doors on a third-floor balcony were inoculated with urediospores there. When the host plants matured (Feb. 7, 1932) the stems bearing teliospores were bagged in tarlatan bags and placed on the surface of damp soil in a box on the balcony. The soil was kept damp. Sixteen weeks later (May 28, 1932) these teliospores were germinating freely and were used in inoculations. This is the shortest rest period that has been observed at Berkeley, Calif. It is not known how long the teliospores can live, but a few collected in the summer of 1931 were still viable in June 1932. According to Pethybridge, Lafferty, and Rhynehart (*42*), teliospores of flax rust can retain viability for 1 year and 9 months.

MYCELIUM

The present cytological study of flax rust has been limited to the haploid generation, fertilization, and the development of aecia, with a brief comparative study of the uredia.

Usually on the eighth day after inoculation, the appearance of the first faint flecks on the leaves indicates that the fungus has made mycelial growth in the intercellular spaces of the leaf and in a few cases has taken the first step in preparation for the development of spermogonia.

The point of entry of the fungus can still be located. The primary hypha, formed by the germinating sporidium when it enters the

epidermal cell, is still present and in some cases has not greatly deteriorated. Plate 1, A_1 and A_2, shows such a primary hypha. The hypha was divided in sectioning, part of it occurring in the section drawn in A_1 and part in A_2. The point of entry was above A_2, *a*. The point *a* in A_2 appears to connect with the point *a* in A_1, and the point *b* in A_2 to connect with the point *b* in A_1. Thus reconstructed, the primary hypha is seen to consist of several uninucleate cells that have branched irregularly. The connections to the surface and to the mycelium beneath the epidermis have been lost as the hypha deteriorated.

Two successive sections of an epidermal cell are drawn in plate 1, B_1 and B_2. Two sporidia germinated and entered the same epidermal cell (pl. 1, B_1, *a*, *b*), giving rise to two interwoven and richly branched primary hyphae. The branches (B_1, *c*, *d*, *e*, *f*; B_2, *a*, *b*, *c*, *d*) extend to the inner face of the epidermal cell, where they give rise to subepidermal mycelium (not included in the drawing). The primary hyphae and their branches are composed of uninucleate cells, so far as noted.

Although the primary intracellular growth of flax rust is composed of uninucleate cells, even a cursory survey of the later intercellular mycelium of these same infections shows the presence of numerous cells containing more than one nucleus. A closer study of the mycelia of a number of 8- and 9-day infections proves that this is typical of the haploid mycelium of flax rust. As the hyphae wind in and out through the irregular intercellular spaces of the mesophyll, it is only an occasional cell in which one can see clearly the two septa delimiting it. A count has been made of 100 such cells from 8-day infections. Of these 100 cells, 34 are uninucleate, 56 are binucleate, 7 are trinucleate, and 3 are quadrinucleate. A similar count of 100 cells found in five 9-day infections gives 25 uninucleate, 66 binucleate, and 8 trinucleate cells, and 1 quadrinucleate cell. No haploid infection of flax rust has been observed with ordinary uninucleate mycelium.

It is rare to see within the limits of one section a succession of cells of a branching system of hyphae. The best example found in the survey of 8-day infections is shown in plate 1, *C*. Some of the cells are uninucleate and some contain two nuclei. If the two minute dots at *a* are nuclei, the cell containing them has five nuclei of different sizes. Plate 1, *D*, shows a detail from a 10-day infection. Binucleate cells prevail here.

In plate 1, B_1 and B_2, two primary hyphae occur in the same epidermal cell and each gives rise to mycelium in the air spaces beneath the epidermis. Plate 1, *E*, represents a bit of that mycelium. Plate 1, *C* and *D*, shows that when growth is unrestricted in a large intercellular space a branch will form an acute angle with its parent hypha. This serves to indicate the direction of growth of a hypha. On that basis, in plate 1, *E*, two hyphae entered the intercellular space from opposite corners (indicated by arrows). The

EXPLANATORY LEGEND FOR PLATE 1

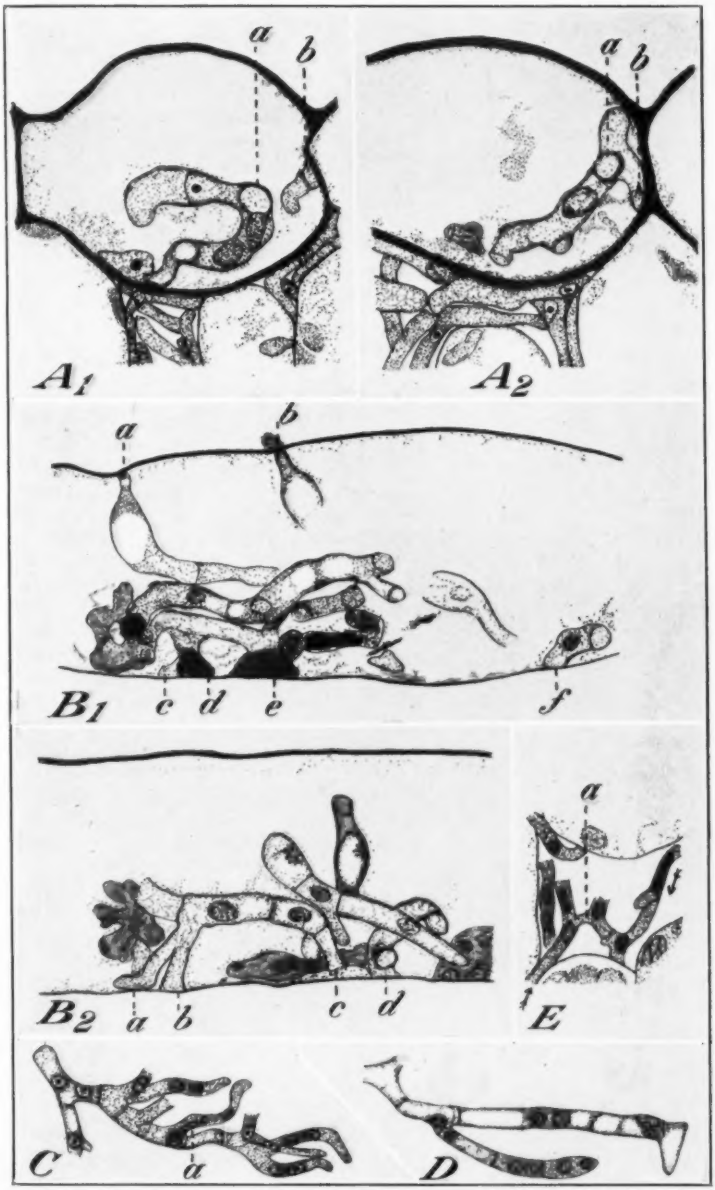
A_1 and A_2.—Successive sections through remnant of primary hypha in epidermal cell of 8-day infection *a*, *b*, Points where the hyphae in A_1 connect with A_2. ×1,020.

B_1 and B_2.—Successive sections through epidermal cell of 8-day infection containing 2 primary hyphae: B_1, *a*, *b*, Points of entry; B_1, *c*, *d*, *e*, *f*, and B_2, *a*, *b*, *c*, *d*, points of exit from the epidermal cell. ×1,020.

C.—Detail of vegetative haploid mycelium from 8-day infection; multinucleate cell at *a*. ×1,020.

D.—Detail of vegetative haploid mycelium from 10-day infection. ×1,020.

E.—Detail of 8-day infection, showing possible hyphal fusion at *a*. ×1,020.



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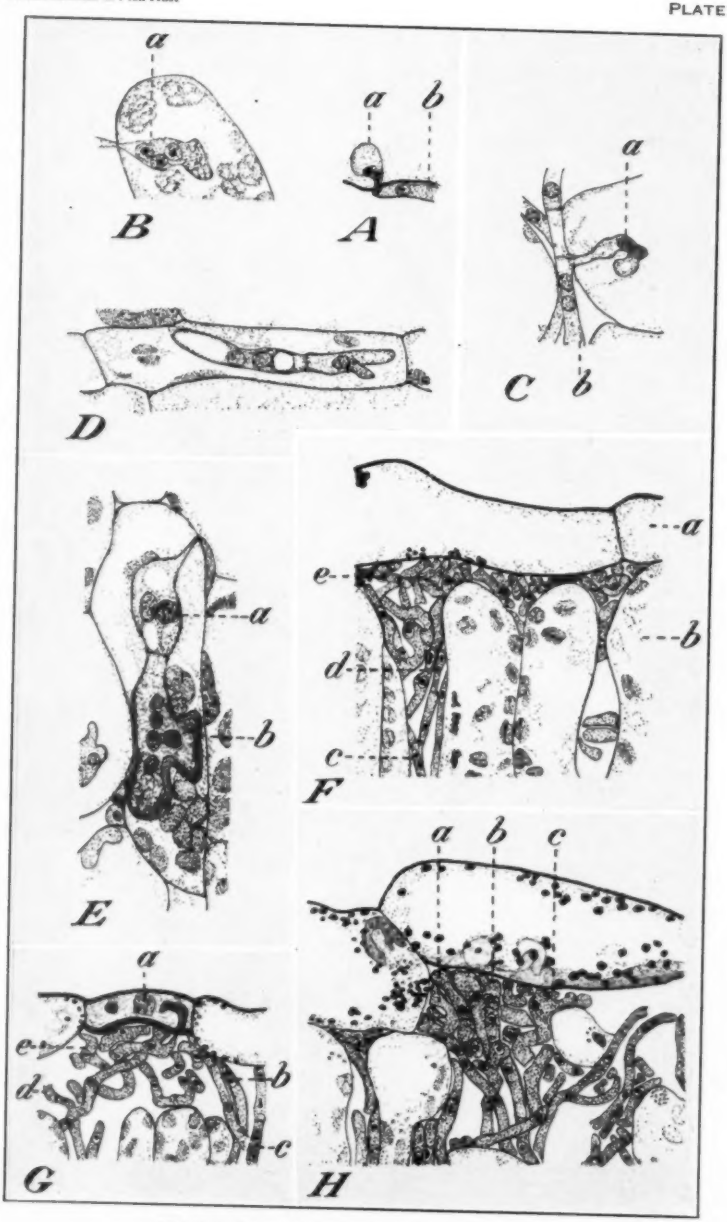

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configuration of hyphae here is difficult to explain except as an anastomosis between the two hyphae at *a*. Of course it cannot be proved that the two hyphae belong to different mycelia, for the two closely interlaced mycelia here are indistinguishable in appearance.

Haustoria of flax rust are as variable in nuclear content as is the mycelium producing them. A young haustorium may contain a single nucleus. Very early, however, it becomes multinucleate. In plate 2, *A*, the young haustorium (*a*) contains one nucleus, and a second is just entering from the hypha (*b*) outside the host cell. The haustorium (*a*) in plate 2, *B*, contains three nuclei. In plate 2, *C*, is another young haustorium at *a*. In this instance the haustorium does not arise from the apical end of the cell (*b*) that produces it. In plate 2, *D*, is shown a 2-cell haustorium, 1 cell containing 2 nuclei and the other 1 nucleus. Under very favorable conditions haustoria increase greatly in size and their nuclei multiply proportionately. In plate 2, *E*, the large 2-cell haustorium (*a–b*) contains 7 nuclei, 2 in 1 cell and 5 in the other.

SPERMOGONIA

Usually on the ninth day following inoculation or rarely on the eighth, mycelial hyphae grow up between palisade cells to the epidermis. This may occur at narrowly localized spots or it may be more widespread. Plate 2, *F*, from a 9-day infection, shows an early stage in this process. Hyphae have grown up between palisade cells and forced a passageway between the epidermal and palisade layers and have formed a continuous mat of mycelium between the epidermal layer (*a*) and the palisade layer (*b*). The binucleate cells common to the mycelium are still present (pl. 2, *F*, *d*, *e*) but less abundant. In the hypha (*c*) 3 successive cells contain 2, 1, and 2 nuclei, respectively. A count of 100 cells in these reproductive areas gives 85 percent of uninucleate cells, showing that the mycelium here is much more closely septate.

These subepidermal hyphae congregate in the substomatal air chambers as shown in plate 2, *G*. At *a* is the guard cell of a stoma and beneath it a loose weft of hyphae. Here, too (*b*, *c*, *d*, *e*) are cells with more than one nucleus. These subepidermal hyphae branch rapidly, the newly formed tips flattening themselves against the inner face of the epidermis (pl. 2, *H*, *a*, *b*, *c*) and pushing it upward, widening the space between palisade and epidermis.

Further growth centers on the stomata. Hyphae of the substomatal chamber grow upward and hyphae of adjoining areas slant in toward the stoma. The result (pl. 3, *A*, from a 9-day infection) is a small mound of mycelium with a stoma (*a*) at its apex. This is the young spermogonium. At this stage the slender, tapering hyphae centered on the guard cells consist of 2 or 3 short cells, usually uninucleate, sometimes binucleate. They may remain unbranched (pl. 3, *B*) or may branch once (pl. 3, *C*), twice (pl. 3, *D*), or, more rarely, several times.

EXPLANATORY LEGEND FOR PLATE 2

A.—Young haustorium (*a*) arising from hypha (*b*); 8-day infection. ×1,020.
B.—Larger haustorium (*a*) with 3 nuclei. 8-day infection. ×1,020.
C.—Binucleate haustorium (*a*) from hypha (*b*); 8-day infection. ×1,020.
D.—Two-cell haustorium from 8-day infection. ×1,020.
E.—Large haustorium with 2 nuclei in one cell (*a*) and 5 in the other (*b*); 8-day infection. ×1,020.
F.—Mycelial mat preparatory to the development of spermogonia forming between epidermis (*a*) and palisade (*b*). Binucleate cells at *c*, *d*, and *e*; 9-day infection. ×640.
G.—Hyphae accumulating beneath the stoma (*a*). Binucleate cells at *b*, *c*, *d*, *e*; 9-day infection. ×640.
H.—Rapid branching of hyphae in subepidermal mat at *a*, *b*, *c*; 9-day infection. ×640.

Sometimes a spermogonium is isolated, but more commonly several occur near together. In plate 3, *E*, is shown at low magnification a cross section through a leaf bearing a typical 9-day infection. It is drawn in outline only, with the fungus shaded to bring it out more clearly. A continuous mat of hyphae has formed under the upper epidermis and the young spermogonia are beneath the stomata at *a*, *b*, and *c*. There is no suggestion of the formation of aecia.

In the stage of development of the spermogonium shown in plate 3, *A*, the hyphae centered upon the stoma look alike. Later development shows, however, that they will not all have the same function. In plate 3, *F*, is shown a slightly older spermogonium from a 9-day infection. The short, tapering, pointed hyphae with tips on the line from *a* to *f* will become the spermatiophores. Among these are longer, fragile-looking hyphae, the paraphyses (pl. 3, *F*, *d*). Two or three of these (pl. 3, *F*, *b*) have grown out to the cuticle between the guard cell (*c*) and the next epidermal cell. Just beneath the epidermis and growing parallel to it are still other hyphae (*e*) which are neither paraphyses nor spermatiophores.

The newly matured spermogonium uses the stoma at its apex as an ostiole. Paraphyses grow out through the stomatal aperture and the first-formed spermatia ooze through the same opening. In plate 4, *B* (drawn in outline only), is shown the apex of a spermogonium with the guard cells of the stoma (*a*, *d*) seen in cross section, the paraphyses (*c*) between the guard cells, and spermatia (*b*) outside. In plate 4, *A* (from an 11-day infection), is the apex of another spermogonium with the stoma (*c*) shown in longitudinal section. Spermatia (*a*) have oozed out and paraphyses (*b*) have grown out through the stomatal aperture. In both of these the epidermis is still intact.

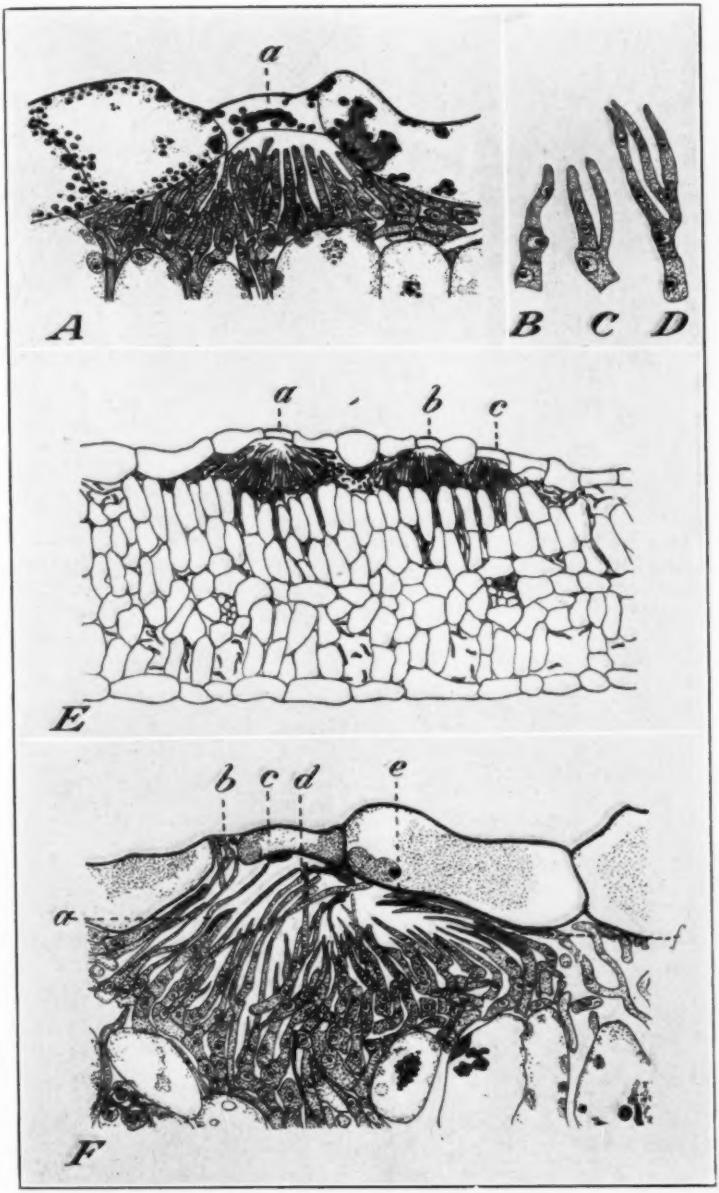
If the stoma still has the power to open and shut, the paraphyses may be nipped off when the stoma shuts. In plate 4, *D*, from a 13-day infection, is shown at high magnification the apical stoma of a spermogonium. Outside are short hyphae that may be broken fragments of paraphyses; an alternative possibility, to be discussed later, is that some of the shorter hyphal bits are germinating inward-growing spermatia.

The epidermis covering a spermogonium is out of its natural food relations with the rest of the leaf. Moreover, it is under tension, as the growing spermogonium beneath lifts and stretches it. Sooner or later the weakened epidermis breaks under the growing pressure from within. The guard cells and sometimes adjoining epidermal cells are sloughed off. In plate 4, *C*, the break has come between the end of the guard cell (*c*) and the next epidermal cell (*a*). Paraphyses (*b*) are pushing out into the opening. Plate 4, *E*, shows an open, active spermogonium from an 11-day infection. A few of the paraphyses (*a*, *b*) have branched, which gives them an antlered effect.

In the case of the larger subepidermal mats on which several spermogonia arise, as in plate 3, *E*, *a*, *b*, *c*, the epidermis over the whole area may be lost, and the separate spermogonia may become merged into one, as in plate 5, *A*, *a*.

EXPLANATORY LEGEND FOR PLATE 3

A.—Young spermogonium forming beneath the stoma (*a*); 9-day infection. ×640.
B, *C*, and *D*.—Details showing branching of spermatiophores; 10-day infection. ×1,020.
E.—Diagram of 9-day infection, showing young spermogonia beneath the stomata (*a*, *b*, and *c*). ×160.
F.—Older spermogonium centered on the stoma at *c*: Tips of spermatiophores along the line *a*–*f*; paraphyses at *b*, *d*; sterile cells at *e*; 9-day infection. ×640.



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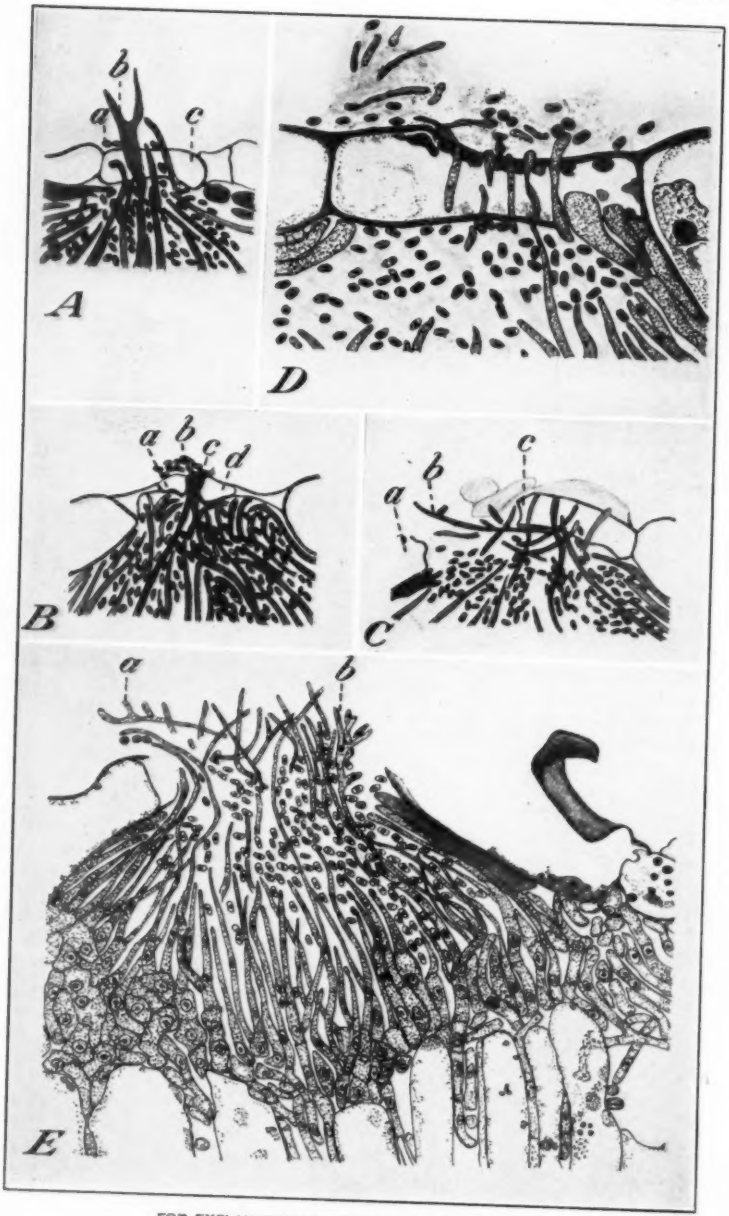

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Spermogonia form at the lower surface of the leaf somewhat later than at the upper (pl. 5, *A*, *b*). In a count of 100 spermogonia in 12-day infections, 72 opened at the upper surface and 28 at the lower.

GREENHOUSE EXPERIMENTS ON HETEROTHALLISM

Melampsora lini is heterothallic. The isolated haploid mycelium, to which no spermogonial exudate is brought from another and different infection, forms spermogonia but cannot produce aeciospores.

In one experiment a pot of seedlings was inoculated in the greenhouse and 34 well-isolated infections developed. The plants were not handled and were covered with a light cage covered with tarlatan to prevent the access of insects. In watering, care was taken not to wet the plants nor to touch them. On the thirty-second day after inoculation 32 of the infections were still sterile (i.e., had produced spermogonia but no aecia) and 2 (about 6 percent) were bearing aeciospores. It is, of course, possible that these 2 developed the sporophyte generation homothallically, but this cannot be taken for granted. Transfer of exudate may have occurred through the agency of a chance insect visitor or by accidental direct contact between infections during a wind when the greenhouse ventilators were fully opened. Moreover, there is always a chance that during inoculation two sporidia alighted so close together on a leaf (pl. 1, B_1, B_2) that the mycelia produced by them grew into a single rounded infection that could not be recognized macroscopically as a double infection.

In another experiment 4 flax seedlings were grown in one pot. When 7 or 8 inches high the plants were inoculated in the greenhouse. Fourteen days later, when the infections were all in evidence, the double infections (2 in contact) and the multiple infections (several confluent) were removed from all 4 plants. Two of the plants were then left undisturbed. On the other 2 plants an attempt was made to transfer exudates back and forth miscellaneously among the infections, toothpicks wet in rain water being used for the purpose. To each infection was brought exudate from several others. Counts were made 2 weeks later, or 28 days after inoculation. On the 2 plants with mixed exudate there were 61 infections. Of these, 41, or 67 percent, produced aeciospores, while 20, or 33 percent, remained sterile. On the 2 plants used as controls there were 128 isolated infections. Of these, 8, or 6 percent, became fertile, while 120, or 94 percent, remained sterile.

As may be seen from this, the attempt to fertilize 100 percent of the infections by mixing their exudates was only a partial success. Flax rust produces an exceptionally small amount of exudate and the period during which it is formed is brief. Moreover, among infections of the same age, some will bear the minute, clear drop of fluid, while others are dry. Although not wholly satisfactory, the data suffice to prove that spermatia function in effecting fertilization in flax rust.

EXPLANATORY LEGEND FOR PLATE 4

A.—Diagram showing apical stoma (*c*) of spermogonium, with spermatia at *a* and paraphyses at *b*; 11-day infection. ×640.

B.—Guard cells (*a*, *d*) of apical stoma of spermogonium, with spermatia at *b* and paraphyses at *c*: 11-day infection. ×640.

C.—Diagram showing rupture of epidermis over spermogonium between guard cell (*c*) and epidermal cell (*a*), with paraphyses growing out at *b*; 11-day infection. ×640.

D.—Apical stoma of a spermogonium, showing spermatia and hyphal bits outside; 13-day infection. ×1,020.

E.—Mature, fully opened spermogonium, with branched paraphyses (*a*, *b*) extruded; 11-day infection. ×640.

Experiments were carried out to learn how early and how late in the life of an infection fertilization can take place. Under greenhouse conditions at Berkeley, the infections first became visible as vague flecks 8 days after inoculation. Beginning with the eighth day, exudate was transferred to infections of different ages including those 8, 10, 13, 14, 20, 25, 32, 35, and 40 days of age. The results were positive from the eighth day to the thirty-second day, most abundant between the eighth and twentieth, and rare after that. Only one infection 32 days old developed aecia when spermatia were applied to it.

As stated before, infections do not become visible macroscopically until 8 days of age, yet 14 out of 23 such infections developed aecia after spermatia had been transferred to them. It is not likely that fertilization occurs often in nature at the 8-day stage, however, for since the infection is inconspicuous and is producing no spermogonial exudate of its own, there would be nothing to attract the insect carrier of spermatia.

The question arises as to whether there are 2 sexual groups or more than 2. In an attempt to determine this, three pots of seedlings with widely scattered infections were used. The infections were paired, i.e., exudate was transferred back and forth between each two infections. Toothpicks moistened with rain water were used for this—a fresh toothpick for each pair. Two or three infections in unfavorable locations died before the count was made. Two others developed telia direct, without forming aeciospores, and as it was not known just what happened here, these also were excluded from the count. Apart from these, the count showed 41 sterile and 44 fertile infections.

If there are only 2 "sexes" it would be expected that half of the matings would be between infections of the same sex and would remain sterile, while the other half would be between infections of opposite sex and would prove fertile. The result, 41 to 44, is a fairly close approximation to the 50 : 50 ratio expected. Although this does not prove, of course, that there are only 2 sexes in *Melampsora lini*, it may be regarded as an indication that only 2 were present in the material used in this experiment.

NONAECIAL INFECTION

Material was available for the study of the later history of the isolated, monosporidial infection. Reference has already been made to the outline drawing of the small 12-day infection in plate 5, *A*. The mycelium permeates the intercellular spaces for a short distance. A large, open, composite spermogonium (pl. 5, *A*, *a*) has formed at the upper surface of the leaf and a younger one (*b*) is developing at the lower surface. There is no hint of the formation of an aecium.

The condition 5 days later (17-day infection) is represented in plate 5, *B*. The infection has spread (only a portion of it is represented) and a massive subepidermal growth (pl. 5, *B*, *b*, *c*) has developed at both the upper and lower sides of the leaf. The spermogonia (pl. 5, *B*, *a*, *d*) are still actively producing spermatia.

Plate 5, *C*, represents the corresponding portion of a 35-day sterile infection (at which age the majority of the infections are dead). The spermogonia (*a*, *b*) are inactive and are dead or dying. The heavy pseudoparenchyma about the spermogonia often extends under the epidermis from one spermogonium to the next. Possibly this is the

equivalent of the haploid aecium of the other rusts studied—nothing else has been found that could be so interpreted. However, there is no organization here that suggests an aecium, and certainly no move looking toward spore formation.

SPERMATIA

Experiments already cited have proved that spermatia smeared upon the surface of an infection enter in some fashion and become effective in initiating the sporophyte generation. Attempts have been made to determine how this takes place.

A search was made for hyphae that had reached the surface of the leaf and that might therefore serve to receive spermatial nuclei. In a section-by-section survey of four 8-day, four 9-day, four 10-day, and two 12-day infections no case was noted in which a hypha pushed out between two epidermal cells to the surface at either the upper or the lower epidermis. Nor was any case found of an intracellular hypha that had pushed out to the surface. Haustoria form freely in epidermal cells, but they usually keep within the inner part of the epidermal cell. Occasionally one reaches the outer wall of the epidermal cell, but never has one been seen to push through and become a subcuticular hypha.

Wherever a subepidermal mat of hyphae forms, the mycelium is pressed against the inner face of the stomata, but with rare exceptions it is only at spermogonia that hyphae actually pass into and through the stomatal aperture. In considerably older infections, in which the epidermis covering a subepidermal mat has become much weakened, a hypha may push out between its cells, but even here it is rare. It is only in older spermogonia that a break in the epidermis exposes the fungus freely to the outer air. If fertilization takes place in *Melampsora lini* by the direct entrance of spermatial nuclei into hyphae, it must take place at the spermogonia.

It was at first thought that the fragile, thin-walled paraphyses extruded through the apical stoma of the spermogonia might serve as receptive hyphae, but no evidence of this has been found. Moreover, an experiment proves that fertilization can take place in the absence of paraphyses. As stated in the description of the greenhouse experiments (p. 772), exudate was smeared on the surface of 23 infections 8 days old. Infections first become visible macroscopically on the eighth day as faint greenish flecks, a shade lighter than the rest of the leaf. In the experiment just cited spermogonia had not developed, yet 14 out of these 23 infections showed open aecia 3 or 4 days after the transfer. The spermatia in this experiment did not enter through spermogonia nor through receptive hyphae between epidermal cells, yet the results indicate that they did enter.

A cursory study of later material (pls. 11, 12, 13) shows unmistakably that cell fusions occur regularly in the young aecia. It is unlikely that there would be sexual fusion at two stages in the same life cycle. These considerations indicate that spermatia somehow enter without fusing with hyphae at the time of entrance. The search for the mode of entrance of spermatia recommenced on this basis.

For this study several hundred 13-day infections that had been grown in the greenhouse were used. Spermatial exudate was mixed back and forth, bringing spermatia to each infection from several

others and applying it to both the upper and under leaf surfaces. The infections were fixed, a few at a time, at short intervals after the exudate was transferred. Some were fixed 15 minutes after the exudate was transferred, then 30 and 45 minutes after, then 1 hour, 1½, 2, 2½, 3, 4, and 5½ hours, 1 day, 2 days, and 3 days after. The following studies were made from this material.

Safranine and methylene blue, the combination stain used in the early part of this work, do not bring out clearly the details within the spermatium. For this purpose, iron haematoxylin, counterstrained with Congo red, has been found satisfactory.

The spermogonia form a feasible avenue of entrance (in infections old enough to have mature spermogonia), and it may be that spermatia placed on an ostiole utilize the sirupy liquid of the spermatial exudate as food and grow into delicate hyphae that enter the spermogonium and then pass on through into mesophyll tissues. The spermogonium shown in plate 6, *A*, was fixed 5½ hours after spermatia were transferred to it. Some of the hyphae that pass through the stoma (*b–g*) are probably paraphyses growing outward. Perhaps some of the exceedingly fine hyphae, as at *e*, are germinating spermatia growing inward. At *a*, *c*, *d*, *f*, and *h* are what appear to be germinating spermatia, each with a nucleus near its center. It is, however, seldom possible to trace any hypha for any distance in a section, and so far no way has been found to distinguish between a fine paraphysis growing outward and a delicate hypha, supposedly of spermatial origin, growing inward.

A highly magnified detail from an open spermogonium is shown in plate 6, *B*. Ordinarily a spermatium forms at the tip of a tapering spermatiophore as in plate 6, *B*, *d*. Occasionally a spermatiophore grows out into a finely attenuated thread (pl. 6, *B*, *c*), at the tip of which a spermatium forms. A spermatium connected with a delicate thread as at plate 6, *B*, *b*, might be mistaken for a germinating spermatium growing inward if it were not occasionally possible to trace such a thread from the spermatiophore below to the spermatium, as in plate 6, *B*, *a*.

Spermatia on the leaf surface near ostioles were examined in the hope of finding some that germinated, grew to the ostiole, and entered. Plate 6, *C*, shows a group of germinating spermatia highly magnified (×2,250). Germination up to this point has been seen frequently. Each spermatium is presumably surrounded by at least a thin film of the liquid of the exudate, but when the plant bearing the infections is kept under ordinary conditions this would soon dry and stop the process of germination.

Since it has been proved that spermatia can enter a leaf when smeared on the surface of a young infection prior to the formation of its own spermogonia, a study was made of spermatia scattered along the surface of the leaf.

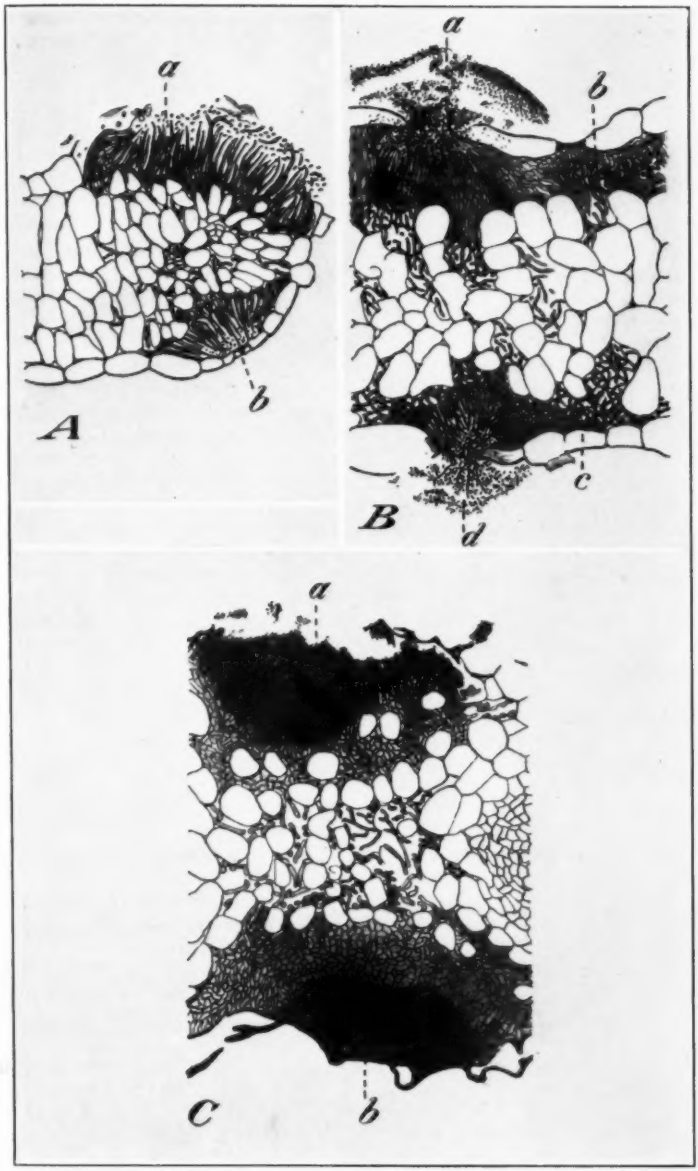
It was thought that spermatia located by chance on a stoma adjoining an infection might germinate and grow in through the stomatal aperture. In spite of prolonged search, no such case has been found. It seems reasonable to expect it, but if it occurs at all

EXPLANATORY LEGEND FOR PLATE 5

A.—Diagram of 12-day sterile infection with spermogonia at *a* and *b*. ×160.

B.—Diagram of 17-day sterile infection, with active spermogonia at *a* and *d*, and sterile subepidermal pseudoparenchyma at *b* and *c*. ×160.

C.—Diagram of 35-day infection, with inactive spermogonia at *a* and *b* subtended by massive dying pseudoparenchyma. ×160.



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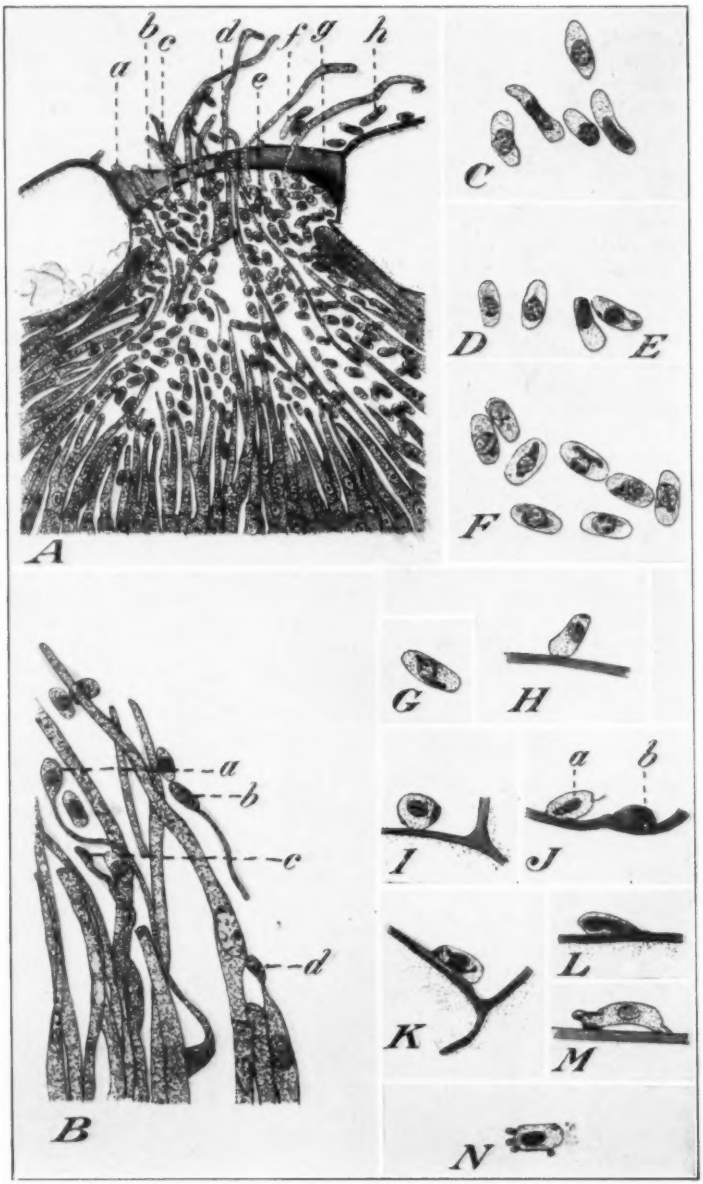

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under the conditions of this experiment it is rare. The minute spermatium contains the very minimum of food and water, which perhaps would not suffice for even this limited growth.

Lastly, attention was focused on spermatia found on the other epidermal cells. Such spermatia undergo a regular series of changes. The newly formed spermatium, before its extrusion through the ostiole (pl. 6, *D*), is a little capsule-shaped cell with a central nucleus and uniform cytoplasm. Shortly after it reaches the surface (pl. 6, *E*, *F*, *G*) some darker-staining material forms at one or more points on the nuclear membrane and extends out into the cytoplasm. It is not clear whether this material is being extruded from the nucleus or whether, as seems more likely, it is arising in the cytoplasm at the nuclear membrane under the influence of the nucleus. At whatever points on the membrane it may form, it tends to move out to the ends of the cell.

Plate 6, *H*, *I*, and *J*, *a*, shows spermatia in contact with the wall of an epidermal cell. The darker-staining matter has reached the outer wall of the spermatium. Some spermatia in this and in subsequent stages were found at the upper epidermis and some at the lower. For convenience in making comparisons, however, the drawings are oriented on the plate as if the spermatia were found at the upper surface of the leaf.

A spermatium may land upon the surface of the leaf in any position, but it soon tends to flatten down against the cuticle. In plate 6, *J*, *a*, this flattening has begun. In plate 6, *K*, the spermatium is well flattened down onto the leaf surface and the darker staining matter is aggregating at one end in contact with the leaf. In plate 6, *L*, the spermatium has changed shape, the dark-stained matter appears to have been extruded from the cell at the right end, and at the center a new connection has been formed between the spermatial nucleus and the leaf surface. Plate 6, *J*, *b*, may be an oblique view of this stage.

It sometimes happens that a spermatium at this stage is ripped loose from its anchorage to the leaf. In plate 6, *M*, the spermatium is partly loosened; the ends of the cell remain attached to the leaf. In plate 6, *N*, the spermatium is torn loose and is turned over. Five little droplets of material have been extruded at the ends of this spermatium. It is possible that the dark-staining material, which formed at the nuclear membrane, then moved to the ends of the cell and passed out through the cell membrane, aids in anchoring the spermatium to the leaf.

Evidence that spermatia penetrate the outer epidermal wall of the leaf has been found in material fixed 1½ hours after spermatia were transferred to it. Penetration of the outer epidermal wall may take

EXPLANATORY LEGEND FOR PLATE 6

A.—Upper part of spermogonium, fixed 5½ hours after spermatia had been transferred to it: *b-g*, Apical stoma; *a*, *c*, *d*, *e*, *f*, *h*, possibly germinating spermatia beginning to grow inward through spermogonium. ×1,020.

B.—Detail of open spermogonium, showing spermatium on short spermatiophores at *d* and spermatia on long stalks at *a*, *b*, *c*. ×2,250.

C.—Germinating spermatia near ostiole, 2½ hours after spermatia had been transferred to the infection. ×2,250.

D.—Spermatia within the spermogonium where they were produced. ×2,250.

E, *F*, and *G*.—Spermatia soon after being transferred. ×2,250.

H and *I*.—Spermatia in contact with epidermal wall 2 hours after transfer. ×2,250.

J.—Spermatia 2 hours after transfer: *a*, Spermatium flattening down onto leaf; *b*, oblique view of more advanced stage. ×2,250.

K and *L*.—Spermatia flattened onto epidermal wall, 2 hours after transfer. ×2,250.

M.—Spermatium torn loose partly, attached at ends, 2 hours after transfer. ×2,250.

N.—Spermatium ripped loose from epidermis, showing small globules extruded at ends, 2 hours after transfer. ×2,250.

place at the center of the spermatium (pl. 7, *A*) or at one end (pl. 7, *C*). Plate 7, *B*, *a* and *b*, shows what probably are end views or oblique views of the same stages. In *C* the first protoplasm from the spermatium has entered, pushing the plasma membrane of the epidermal cell ahead of it. Plate 7, *E*, may be a late stage in entry, but, since it was not possible to trace a connection between *a* and *b*, its status remains uncertain. In plate 7, *D*, *a*, is another stage of somewhat doubtful status.

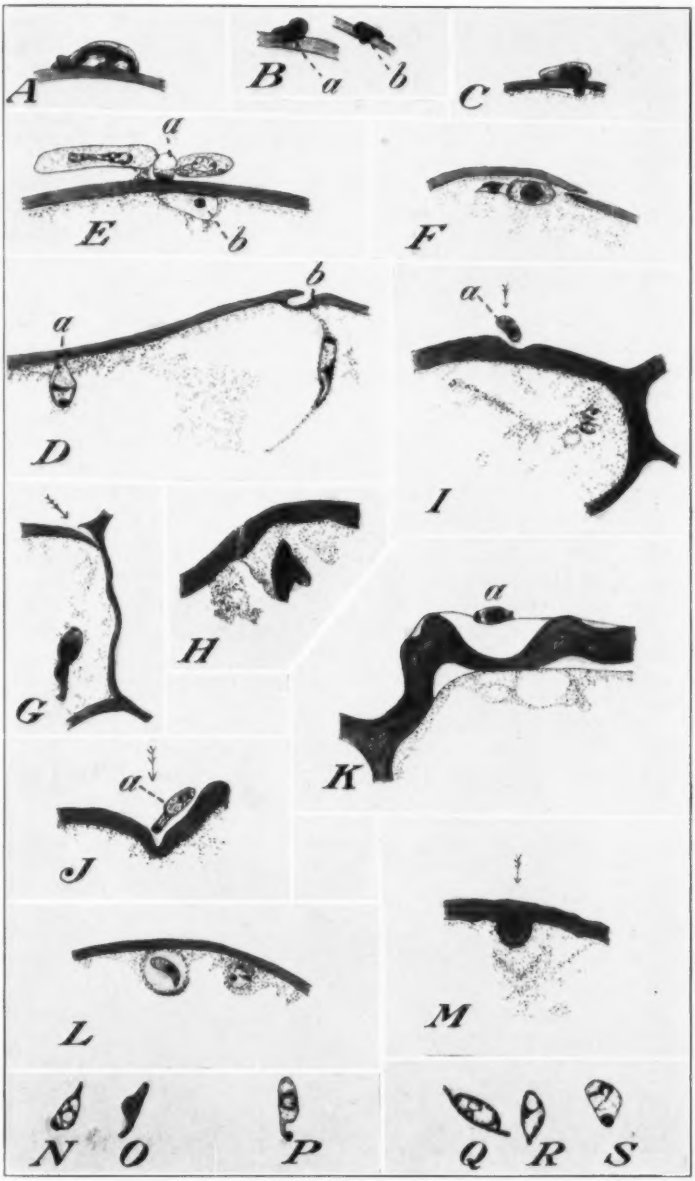
Plate 7, *F*, shows a newly entered spermatium. Careful focusing shows a minute diagonal opening through the wall behind it. Plate 7, *D*, *b*, shows a rather broad diagonal opening in the outer wall of the epidermal cell and inside is a spermatium that is apparently being transported across the cell on a fine strand of streaming cytoplasm. In plate 7, *G*, the spermatium has moved farther from the diagonal opening in the wall at the joining of the outer wall to the side wall of the epidermal cell, indicated by the arrow. Plate 7, *H*, is similar, except that the beak is at the side instead of at the end of the spermatium.

Search was made in these and similar cases for a discarded membrane left outside the epidermal cell when the spermatium entered. No amount of focusing or adjustment of the lighting has revealed it. The spermatium wall, if left behind, is so delicate and evanescent that it escapes detection. Moreover, the spermatium after entering the cell, still has a visible membrane.

The question arises whether the spermatium enters by mechanical or by chemical means. Some light on this can be gained by a study of spermatia located on walls too heavy to be penetrated. Plate 7, *I*, shows a spermatium on the heavy epidermis of an older leaf. Beneath it the cell wall is thinner, although the cuticle appears intact. In plate 7, *J*, is a more extreme case—the wall is not only thinner at the point nearest the spermatium but it is misshapen. In plate 7, *K*, the wall is warped out of shape for some distance, and the spermatium is lying on the cuticle stretched across the top of the indentation of the wall. Perhaps the cuticle of an older leaf is too tough for the spermatium. In this case (pl. 7, *K*), where the wall is deformed for some distance, it may be surmised that several spermatia were originally present, the rest having been washed off in the processes of fixing and staining. Only an occasional spermatium produces this effect; the rest cause no noticeable deformation of the host wall. It is not clear why this is so. It happens often enough, however, to suggest that the action of a spermatium in entering the epidermal cell is partly due to mechanical force and partly to some secretion—perhaps enzymatic in character—which softens the wall.

EXPLANATORY LEGEND FOR PLATE 7

A.—Spermatium penetrating the outer wall of the epidermal cell, 2 hours after transfer. ×2,250.
B.—Oblique views, *a*, *b*, of spermatia at similar stages. ×2,250.
C.—Spermatium from which the first protoplasm has passed into the epidermal cell, 2 hours after transfer. ×2,250.
D.—Spermatia 1½ hours after transfer: *a*, Possibly late stage in entry; *b*, opening in wall through which spermatium passed. ×2,250.
E.—Group of spermatia: *a*, *b*, Possibly late stage in entry. ×2,250.
F.—Newly entered spermatium with minute passageway in wall behind it, 2 hours after transfer. ×2,250
G and *H*.—Opening in wall and spermatium beneath it, 2½ hours after transfer. ×2,250.
I and *J*.—*a*, Spermatium on surface, with a dent in the heavy wall beneath it. ×1,400.
K.—*a*, Spermatium lying on taut cuticle, with warped heavy wall beneath it, 2½ hours after transfer. ×2,250.
L.—Spermatia in epidermal cell and surrounded by shell of host cytoplasm, 2 hours after transfer. ×2,250.
M.—Possibly walled-in spermatium, 2½ hours after transfer. ×2,250.
N, *O*, *P*, *Q*, *R*, *S*.—Spermatia in epidermal cells, 1½ hours after transfer. ×2,250.
All from 13-day infections fixed after spermatia were transferred to them from other infections.



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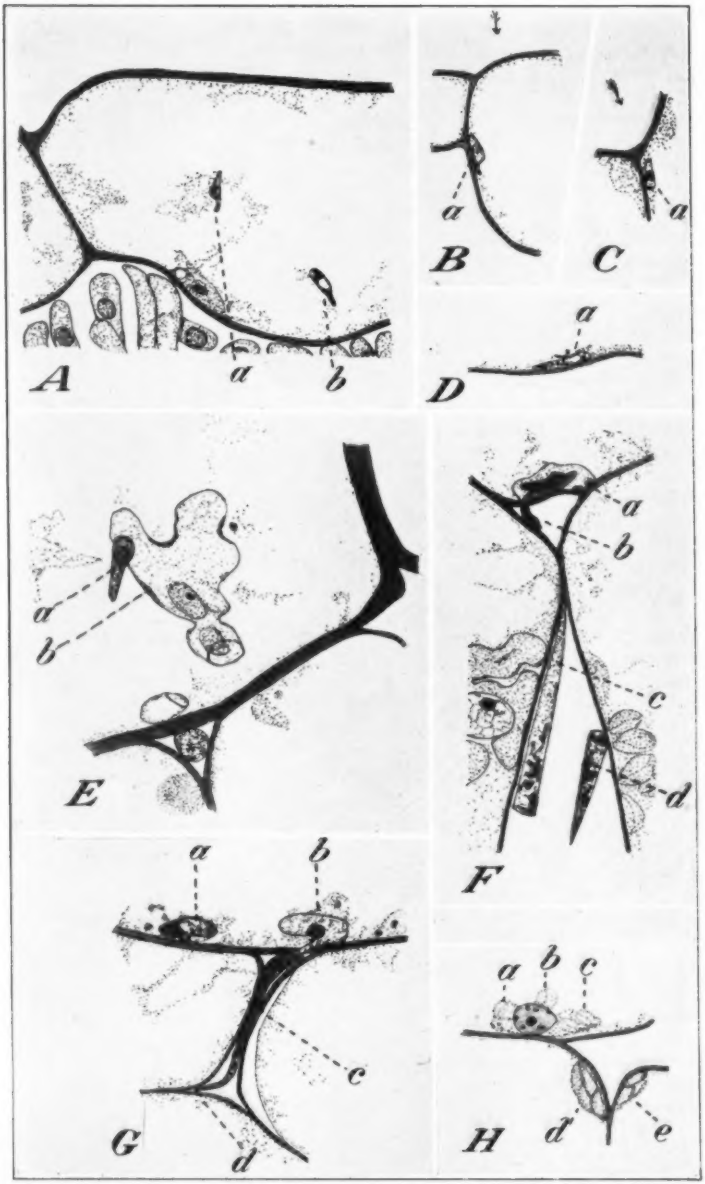

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Every mature infection produces its own spermatia and these are naturally of the same sex as the infection that produced them. The question arises whether the spermatia produced by an infection will enter it. Several days were spent in searching through isolated infections that carried only their own spermatia. So far no case has been found in which such spermatia entered the leaf.

In this connection, it has been interesting to note that spermatia, by chance located out beyond the infection on healthy leaf tissues, do not enter. A few spermatia entered that were located one or two cells beyond the mycelium, but none entered that was farther away. The search for them has not been exhaustive, but the implication of the findings so far (representing a week or so of study) is that the proximity of a mycelium of sex opposite to that of the spermatium is necessary to incite entry.

Plate 7, *L*, shows what appear to be spermatia just within the outer epidermal wall, surrounded by a shell of dense host cytoplasm. In plate 7, *M*, there is no way to prove that the little black-stained mass completely surrounded by a layer of cell wall of the host is the remnant of a spermatium, but a comparison of *L* and *M* indicates that an occasional spermatium is halted just after entry and is walled in.

Plate 7, *N* to *S*, shows spermatia that were found in different parts of epidermal cells. They vary in appearance; in fact, they vary too much. If all these are spermatia, some of them are deteriorating.

Plate 8, *A* to *D*, shows spermatia drawn at lower magnification to include more of the setting. Plate 8, *A*, *a* and *b*, shows two spermatia in the central part of the epidermal cell. Some idea of the relative size of the spermatia and the epidermal nucleus (against the inner epidermal wall) and the subepidermal hyphae can be gained from this. Plate 8, *B*, *a*, and *C*, *a*, shows the spermatia in contact with the side wall of the epidermal cell, and plate 8, *D*, *a*, shows the spermatium lying on the inner wall of the cell.

Spermatia carried passively in the streaming cytoplasm in the epidermal cell may lodge at any point. Commonly they are found near the nucleus of the epidermal cell and may disintegrate there, perhaps digested by the host cell. Sometimes, as in plate 8, *E*, a spermatium (*a*) stops in contact with a haustorium (*b*). In this instance the spermatium has started to grow in toward the inner wall of the epidermal cell.

The smaller the object studied under the microscope the greater is the risk of error in judging it. Spermatia are at just about the lower limit in size. As a precautionary measure, then, all other possible explanations of what has been seen should be considered. What other stainable objects are found in epidermal cells that might be mistaken for spermatia? Such a list would include (1) haustoria, (2) plastids, (3) a primary hypha, (4) translocated loose bits of mycelium deposited by the knife in sectioning, and (5) small rounded granules of unknown composition.

EXPLANATORY LEGEND FOR PLATE 8

A.—*a*, *b*, Spermatia within epidermal cell, 1½ hours after transfer. ×1,400.
B and *C*.—*a*, Spermatia within epidermal cell along its side wall, 1½ hours after transfer. ×1,400.
D.—*a*, Spermatium lying against the inner wall of the epidermal cell, 1½ hours after transfer. ×1,400.
E.—Germinating spermatium, *a*, lodged against haustorium, *b*, in epidermal cell, 1 hour after transfer. ×2,250.
F.—Spermatium (*a*) in epidermal cell, which has given rise to short hypha (*b*). Mycelium at *c*, *d*. ×2,250.
G.—Two spermatia (*a*, *b*) lying against inner wall of epidermal cell, one of which (*b*) has given rise to the intercellular hypha (*c*–*d*), 2½ hours after transfer. ×2,250.
H.—Minute plastids (*a*, *b*, *c*) in an epidermal cell; normal plastids (*d*, *e*) in mesophyll cells. ×1,400.

(1) A full-grown, intact haustorium (pl. 9, *B*, *a*) could not by any stretch of the imagination be mistaken for a spermatium, but one protruding lobe of a haustorium might be cut off in sectioning and appear alone in a cell. Ordinarily, it would be too large, or would have the wrong shape, or would lack a nucleus. Rarely there is a doubtful case. Haustoria in epidermal cells deteriorate rapidly, breaking down into irregular shrunken masses of black-stained matter. These can be recognized at sight, except in rare instances, and do not resemble spermatia.

(2) The great majority of healthy epidermal cells of flax contain no recognizable plastids. Rarely, however, a few minute bodies are found in epidermal cells (pl. 8, *H*, *a*, *b*, *c*) in healthy tissue beyond the infection. They are so transparent that only by reducing the light can their outline be made out. These may be rudimentary plastids. Below in the adjoining mesophyll cells (pl. 8, *H*, *d*, *e*) are drawn two ordinary normal plastids for comparison. It is conceivable that epidermal cells covering an infection might develop plastids, normal or abnormal, as a response to pathological conditions and that the bodies under question in plate 8, *A*, *B*, *C*, *D*, might be such plastids. In that case they should be present in infections of similar age and grown under similar conditions whether spermatia have been transferred to them or not. Some time has been spent searching in the epidermis of isolated infections for bodies comparable to those in plate 8, *A* to *D*, but the results have been negative.

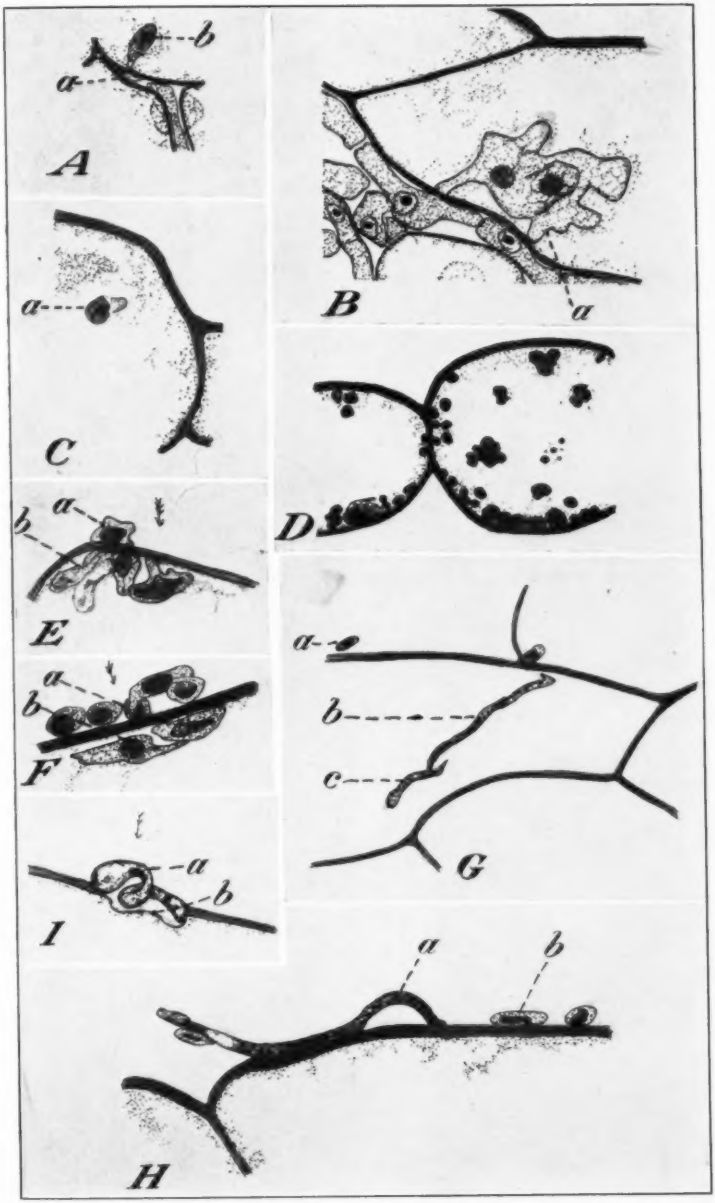
(3) Somewhere in each infection there is or has been in an epidermal cell the primary hypha that inaugurated the mycelial growth. A glance at plate 1, A_1, A_2, and B_1, B_2, shows that there is small chance of confusing it or any remnant of it with a spermatium. Morecver, spermatia can often be found in 20 or 30 epidermal cells, some of which are in the upper epidermis, some in the lower. The primary hypha is in one cell of the upper epidermis.

(4) A bit of mycelium might be caught on the edge of the knife in sectioning and later be deposited in an epidermal cell. This happens occasionally. Ordinarily it can be recognized at sight. Even when, as rarely happens, the translocated bit has nearly the size and shape of a spermatium, it can be recognized. The nucleus in plate 9, *C*, *a*, for example, has exactly the character of the mycelial nuclei in plate 9, *B*. A spermatial nucleus does not have the large central nucleole. Moreover, the microtome knife cuts one way through the leaf—not both ways. It would not deposit mycelial bits in both the upper and the lower epidermis of the same section. Spermatia can be found in both epidermal layers of the same section.

(5) Older flax leaves grown out of doors contain still another type of stainable objects. These are small, rounded granules, simple

EXPLANATORY LEGEND FOR PLATE 9

A.—Young haustorium (*b*) in epidermal cell from hypha (*a*). ×1,400.
B.—Mature haustorium (*a*) in epidermal cell. ×1,400.
C.—Translocated bit of mycelium (*a*) in epidermal cell. ×1,400.
D.—Epidermal cells from flax grown out of doors, showing granular content. ×1,020.
E.—*a*, Spermatium entering epidermis and (*b*) group of spermatia inside and starting to grow; from plant in damp chamber fixed 1½ hours after transfer. ×2,250.
F.—*a*, Spermatia outside, and (*b*) growing spermatia inside of epidermis, from damp chamber 1½ hours after transfer. ×2,250.
G.—*a*, *b*, *c*, Spermatia germinating on surface of epidermis from plant in damp chamber. ×1,020.
H.—Germinating spermatia on epidermis (*a*, *b*) from plant in damp chamber 1 hour after transfer. ×2,250.
I.—Spermatium (*a*) germinating on surface of plant in damp chamber; epidermal wall (*b*) beneath it dissolved; 1 hour after transfer. ×2,250.



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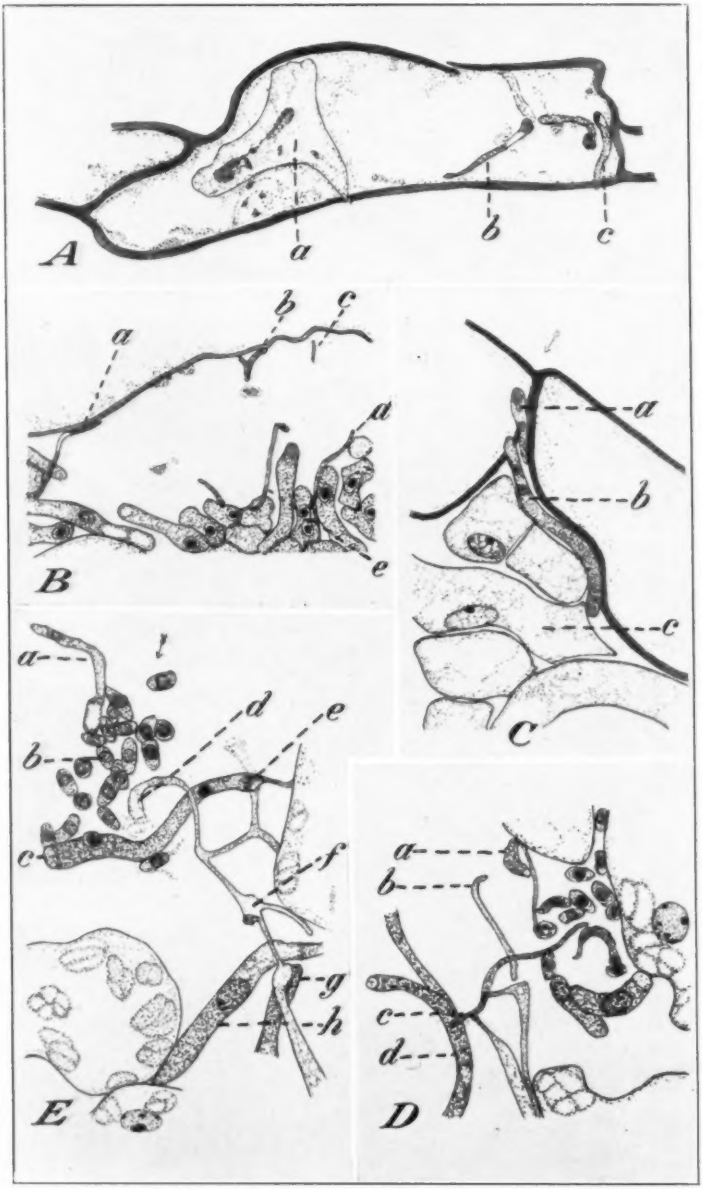

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or compound (pls. 2, *H*; 3, *A*; 9, *D*), which stain a yellowish brown in iron haematoxylin and Congo red and which stain a deep purplish brown in safranine and methylene blue. Sometimes what appears to be the same material is present in a more diffuse form, clouding nearly the whole cell lumen (pl. 3, *F*). Happily this material, whether in diffuse or granular form, is nearly absent from the epidermis of flax grown in the greenhouse. There is no risk of mistaking one of these granules for a spermatium, but the presence of numerous granules in a cell would add greatly to the labor of hunting for spermatia.

In summing up this survey of alternative possibilities, it would appear that, while there is a chance that something else present in an epidermal cell could be mistaken for a spermatium, it is but a small chance.

It may be that many of the supposed spermatia die in the epidermal cells, overcome by the defensive forces of the host. There is also evidence, insufficient as yet, that some survive. These are found in material fixed 2½ and 3 hours after smearing spermatia on the infection.

In plate 8, *G*, at *a*, is drawn a spermatium (×2,250) which is lying against the inner wall of an epidermal cell. A projection on its under side suggests either anchorage to the cell wall or growth preparatory to penetrating that wall.

Plate 8, *F*, shows a spermatium (*a*) and issuing from it a very fine hypha (*b*) that has grown into a triangular chink between palisade cells. Below, at *c* and *d*, are tips of mycelial hyphae. In plate 8, *G*, at *b*, is another spermatium which has given rise to a hypha (*c*), whose growing tip is at *d*. These hyphae are minute, having about one-third to one-fourth of the diameter of ordinary mycelial cells. Perhaps the body of the spermatium, still inside the epidermal cell, absorbs materials from the host to start this growth.

The alternative interpretation at this stage is that an intercellular hypha starting to form a haustorium in an epidermal cell might be mistaken for a spermatium growing out of an epidermal cell into intercellular spaces. In plate 9, *A*, *b*, is a young haustorium of a size comparable to that of a spermatium. So far as noted, the typical young haustorium stands out at right angles to the wall through which it has just passed, whereas the spermatium that is growing out of an epidermal cell lies down flat against the wall it is about to penetrate. The young haustorium, equaling in size a spermatium, has dense contents, and the mother cell outside, which contributed this protoplasm, is depleted thereby (pl. 9, *A*, *a*). The spermatium, after giving up much of its substance to form the hypha outside, is thin in content (pl. 8, *G*, *b*,) whereas the hypha outside is dense (pl. 8, *G*, *c–d*). Moreover, the haustorium mother cell has a large diameter unless cramped for space, while the young spermatial hypha has a small diameter.

For convenience the hyphae of ordinary gametophytic mycelium originating from the growth of a sporidium will be designated "sporidial hyphae", and the growth from a spermatium "spermatial hyphae."

EXPLANATORY LEGEND FOR PLATE 10

A.—Epidermal cell containing haustorium (*a*) and fine spermatial hyphae, *b*, *c*; 1 hour after transfer of spermatia. ×2,250.

B.—Very fine hyphae (*a*, *b*, *c*, *d*, *e*) in space between inner wall of epidermal cell and the subepidermal mycelial mass, 4 hours after transfer of spermatia. ×1,020.

C.—Two spermatial hyphae (*a*, *b*) growing out of epidermal cell into contact with subepidermal mycelium (*c*). ×2,250.

D.—Spermatia within leaf germinating (*a*, *b*, *c*) in contact at *c*, with sporidial hypha (*d*). ×1,400.

E.—Spermatia (*b*) within leaf. Sporidial hyphae (*c*, *h*). Early germination stage at (*a*). Spermatium (*e*) grew and made contacts with sporidial mycelium at *e*, *f*, and *g*. ×1,400.

In a second experiment, spermatial exudate was transferred back and forth among a large number of infections and then the plants bearing them were placed in damp chambers. At short intervals, material was removed from these damp chambers and fixed.

In saturated air there is greater variation in the mode of entrance of spermatia. Some, as before, penetrate the outer wall directly. Plate 9, *E*, shows a little group of spermatia one of which (*a*) is just entering through the outer epidermal wall while four others (*b*) are inside and starting to grow. Plate 9, *F*, represents another group with several spermatia (*a*) outside and two (*b*) inside.

Other spermatia in this wet air germinate, forming delicate hyphae on the leaf surface. Plate 9, *G*, shows at lower magnification a surface view of epidermis. At *a* is an ungerminated spermatium, at *c* a short spermatial hypha, and at *b* a longer hypha. Plate 9, *H*, showing a cross section through epidermis, represents two stages (*a*, *b*) in the germination of a spermatium.

Spermatial hyphae on the leaf surface probably enter. Plate 9, *I*, shows a short, twisted hypha beneath which at *b* the epidermal wall has been dissolved out. The tip of the hypha is ready to enter.

A spermatium which makes some growth before entering would be likely to be anchored to the point of entrance and would not float about in the epidermal cell. If the spermatium is anchored at the point of entrance it starts growing from that point (pl. 9, *E*, *b*). If it is carried about the cell and lodges against a haustorium it may push out a hypha there (pl. 8, *E*, *a*). Plate 10, *A*, shows an epidermal cell containing a large haustorium (*a*) beneath which is a remnant of the host nucleus. At the other end of the cell (*b*, *c*) are 3 or 4 delicate hyphae growing down to the inner side of the cell. A minute opening in the outer wall may indicate the point of entry. One of these hyphae (*c*) is passing through the inner wall of the host cell into the intercellular space.

Twenty-four hours elapse between the placing of spermatia upon the surface of a leaf and the early stages of the formation of aecia. It is of theoretic interest to know how these delicate spermatial hyphae maintain themselves during this period. No one has succeeded in producing independent infections from rust spermatia. How, then, are they nourished here?

Because of the extreme minuteness of these spermatial hyphae, their later history has been difficult to follow. Plate 10, *C*, shows a highly magnified detail of the epidermis and subepidermal mycelium near a spermogonium. This material was fixed 1½ hours after spermatia were transferred to the leaf surface. Two fine hyphae (*a*, *b*), presumably of spermatial origin, are growing out of the epidermal cell. Note the contrast in size between the sporidial hyphae (*c*) and the spermatial hyphae (*a*, *b*).

A search was made for other very fine hyphae in material fixed 4 hours after transferring spermatia to the infection. Some mischance, probably in sectioning, pulled the epidermis loose from the subepider-

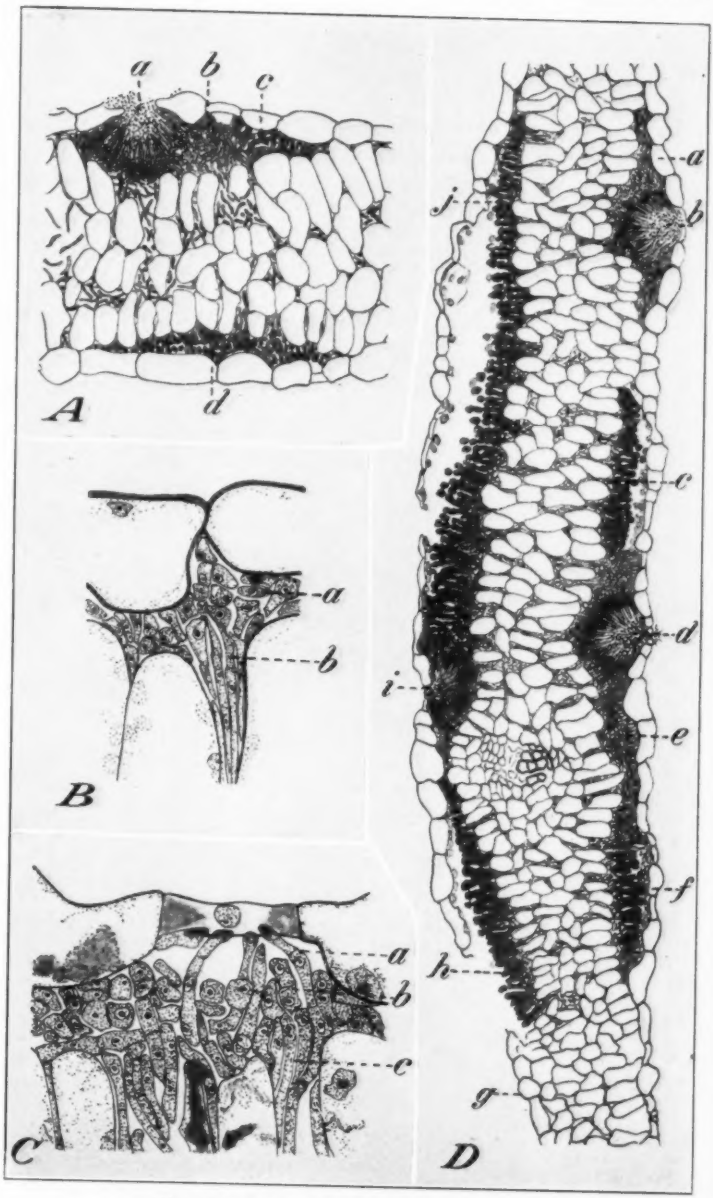
EXPLANATORY LEGEND FOR PLATE 11

A.—Diagram of infection 1 day after spermatia had been transferred to it. Open spermogonium at *a*; pseudoparenchyma at *b*; young aecia at *c* and *d*. ×160.

B.—Detail of very young aecium, showing hyphae pushing out between palisade cells at *b* and giving rise to buffer cells beneath the epidermis at *a*. ×640.

C.—Detail of young aecium (*b*, *c*) overgrowing the rudiment of a spermogonium at *a*. ×640.

D.—Diagram of 16-day infection fixed 3 days after spermatia had been transferred to it. Spermogonia at *b*, *d*, *i*; sterile pseudoparenchyma at *a*, *e*; aecia at *c*, *f*, *h*. *j*; normal leaf tissue at *g*. ×115.



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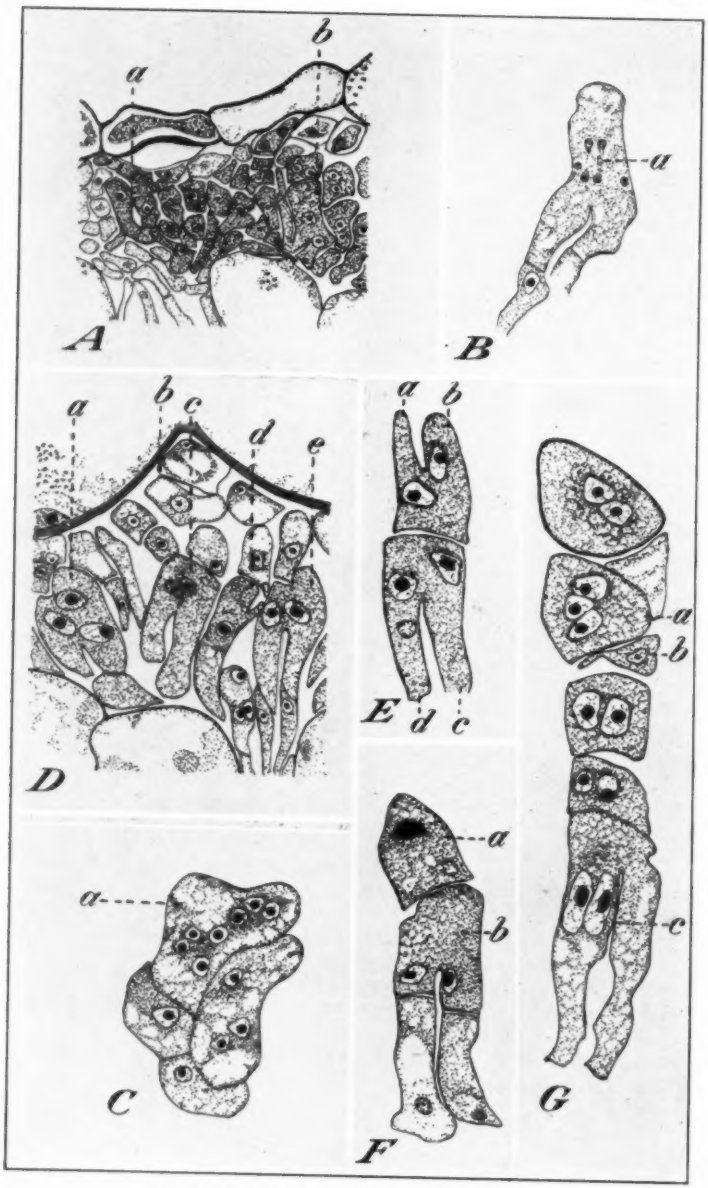

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mal mycelial growth at one point. In the space between the inner wall of the epidermal cell and the coarse hyphae below were very fine hyphae (pl. 10, *B*, *a*, *b*, *c*, *d*, *e*). These were so small that they would have passed unnoticed if they had not projected into a clear space. It may be that spermatial hyphae are fairly common at this stage but that they can rarely be seen.

Some minor injury to a leaf, probably an insect bite, carried a group of fresh spermatia into the interior of a leaf at the margin of a mycelium. Spermatia are shown (pl. 10, *D*, *E*) alongside of sporidial hyphae (pl. 10, *D*, *d*; *E*, *c*, *h*) in the air spaces of the mesophyll tissue. Some of these spermatia germinated. Young spermatial hyphae are shown in plate 10, *D*, *a*, and *E*, *a*. A few have attained greater growth (*D*, *b*, *c*; *E*, *d*). The spermatium in plate 10, *E* at *d*, grew into a fine hypha which branched two or three times. At *g* it made a contact with the growing tip of a sporidial hypha; it swelled and cupped into the end of the hypha and then grew on with increased diameter. At *e* another branch of this spermatial growth met the growing tip of a sporidial hypha, cupped into it, and then grew on. If *f* is a third example, the sporidial hypha was cut off in sectioning. The appearance of these encounters suggests an intimate contact rather than an anastomosis and may be a means of extracting food for further growth.

Soon after this, the spermatial growth becomes indistinguishable in appearance from the mycelium of sporidial origin. The early growth of spermatia is much finer than ordinary hyphae, and it was hoped that the later growth might also be identifiable by smaller diameter of the hyphae or perhaps by having uninucleate cells. Hyphae at this period are variable in size, to be sure, but any hypha can narrow down when passing through a small space and widen out later, and, as it is not possible in sectioned material to trace any one hypha very far, all attempts to prove the existence of two types of mycelium have been unsuccessful As for nuclear content, the vegetative mycelium is still, as it was earlier, composed largely of cells with more than one nucleus. Of 100 cells in a 13-day infection, 31 are uninucleate, 62 binucleate, 6 trinucleate, and 1 quadrinucleate. These ratios do not differ significantly from the percentage of nuclear content of earlier mycelium. Evidently, as soon as spermatial hyphae make contact with food supplies they take on the character of ordinary mycelium.

AECIA

Twenty-four hours after the transfer of exudate to an infection of opposite sex, young aecia are forming at both the upper and lower epidermis (pl. 11, *A*, *c*, *d*), and 3 days after the transfer (pl. 11, *D*, *c*, *f*, *h*, *j*) the aecia are extending rapidly and forming spores.

Plate 11, *A*, shows the general appearance of an infection the day after spermatia had been transferred to it. At *a* is an open spermo-

EXPLANATORY LEGEND FOR PLATE 12

A.—Margin of young aecium: Buffer cells forming at *a*, young spore chains at *b*. ×640.
B.—Conjugate division (*a*) in fusion cell. ×1,400.
C.—Multinucleate cell (*a*) among the young basal cells. ×1,400.
D.—Detail of young aecium, showing decadent buffer cells at *b*, fusion cells at *a*, *c*, and *e*, and a thumblike lobe on the cell at *d*. ×1,400.
E.—Subterminal fusion between the hyphae *ad* and *bc*, followed by division cutting off the 2-prong spore initial *ab*. ×1,400.
F.—Fusion cell (*b*) with first spore initial (*a*). ×1,400.
G.—Spore chain with binucleate basal cell (*c*); trinucleate spore (*a*) with uninucleate intercalary cell (*b*). ×1,400.

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gonium; adjoining it, at *b*, is a subepidermal mass of sterile pseudoparenchyma, and continuous with this at *c* is an early stage in the formation of the aecium; opposite this, at the lower leaf su face, is a second young aecium (*d*). These aecial primordia are readily distinguished from the beginnings of spermogonia by the relative coarseness of the hyphae and their different habit of growth.

Details of young aecia are represented in plate 11, *B* and *C*. From the mycelium of the mesophyll, hyphae grow out between palisade cells (pl. 11, *B*, *b*) and then force a passageway between the epidermis and palisade. These hyphae (p.. 11, *B*, *b*) or branches from them will soon fuse by pairs. Some of them, supposedly, are of sporidial origin and some of spermatial, but no difference in size can be seen at this stage. All are of small diameter at *b* in the narrow space between palisade cells, and all broaden out in the wider space above at *a*.

These hyphae cut off short, usually uninucleate cells (pl. 11 *B*, *a*; *C*, *b*), the buffer or "space-making" cells. Sometimes there is but a single layer of buffer cells, sometimes more (pls. 12, *D*, *b*; 13, *E*, *l*). As many as 3 or even 4 layers may be formed and the number may vary within the limits of the same aecium. These buffer cells soon become separated from the hyphae that bore them and they rapidly deteriorate and die. In newly opened aecia they may still be seen as d sorganized bits (pl. 11, *D*) attached to the inner surface of the loosened epidermis.

Hyphae of young aecia regularly grow perpendicular to the epidermis, or nearly so, without regard to stomata. In plate 11, *C*, at *a*, however, several slender hyphae have centered on the stoma. It would seem probable that these represent an earlier spermogonial rudiment, now overgrown by aecial development.

Hyphae in the aecial primordia are almost completely septate. Counts of the number of nuclei per cell in 100 cells in and adjoining these young aecia show that 83 cells are uninucleate and 17 binucleate.

As the buffer cells become detached and their content becomes thinner (pl. 13, *E*, *a*, *d*, *l*), the hyphae beneath them grow heavier and richer in content (pl. 13, *E*, *b*). These hyphae now pair. The first indications of fusion consist of a lobe on one of the two cells (pl. 13, *A*, *a*) or on both (pl. 13, *B*, *a*). Sometimes a lobe grows out and makes contact with a cell that has already mated (pl. 13, *D*, *a*, *b*, and pl. 12, *D*, *d*, *e*). Once a narrow opening has formed between two cells (pl. 13, *B*, *a*) it broadens rapidly, usually joining the whole upper part of the two component cells (pls. 12, *D*, *a*, *c*, *e*; 13, *E*, *c*, *e*).

In the typical fusion cell, the "2-legged cell" of the literature, the two conjugate nuclei divide side by side (pl. 12, *B*, *a*), giving rise to the first spore initial (pls. 12, *F*, *a*, *b*; 13, *E*, *g*). This initial cell divides again to form the definitive aeciospore (pl. 13, *E*, *k*) and the intercalary cell (*j*). The fusion or "basal" cell (pl. 13, *E*, *i*) grows and divides repeatedly, giving rise to a chain of spores (pl. 12, *G*).

The majority of cases follow the regular development, but irregularities and deviations are common. Rarely, the fusion remains sub-

EXPLANATORY LEGEND FOR PLATE 13

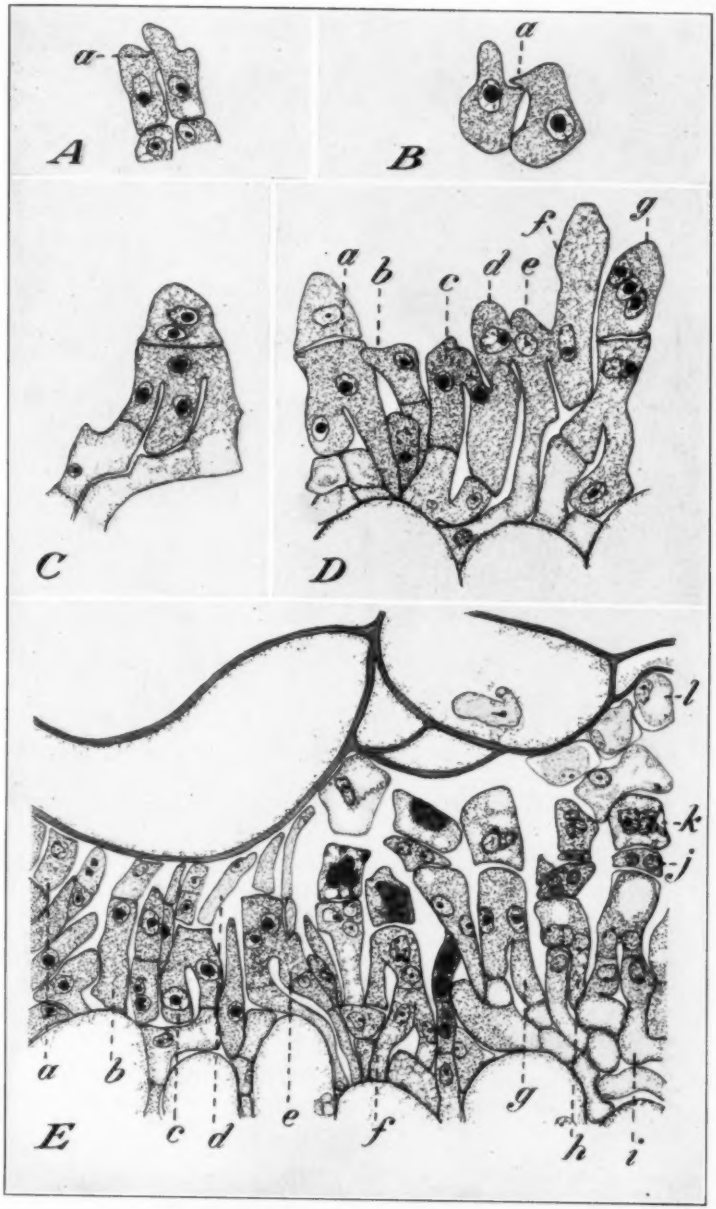
A.—Paired cells about to fuse. Lobe at *a* makes contact. ×1,400.

B.—Early stages in fusion. First opening between the cells at *a*. ×1,400.

C.—Triple fusion. ×1,400.

D.—Detail of young aecium. Lobe of cell at *b* extends to fusion cell, *a*; *c*, *d*, *e*, *f*, joined in multiple fusion; *g*, 2-legged cell cut off trinucleate spore initial. ×1,400.

E.—Portion of section, showing prefusion stages at *b*; buffer cells at *a*, *d*, *l*; fusion cells at *c*, *e*, *f*, *g*, *h*, *i*; and a spore (*k*) with its intercalary cell (*j*). ×1,020.



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terminal. In plate 12, *E*, two hyphae (*ad* and *bc*) fused through the central part of their length and then the first division cut off the 2-prong cell (*ab*).

One of the two cells that form a pair may be already binucleate (perhaps a left-over from the multinucleate mycelium) so that the fusion cell may be trinucleate (pl. 12, *D*, *a*; *E*, *d*). Sometimes the extra nucleus appears to deteriorate (pl. 12, *E*, *d*) and sometimes it passes out into the first spore initial formed (pl. 13, *D*, *g*).

Sometimes three cells fuse (pl. 13, *C*, *E*, *f*). The fusion of the three may be, to all appearances, simultaneous (pl. 13, *C*). Plate 13, *E*, *f*, shows a curious irregularity. Two cells fused, grew, and cut off the first binucleate spore initial. Then this latter was joined by a third cell, grew, and cut off a trinucleate spore initial. Presumably the later spores to be formed in this series would have been trinucleate. Chains of trinucleate spores are not rare (pl. 13, *E*, *h*). Sometimes the cell fusions are more intricate, connecting 5 or 6 cells at different focal depths in the section into a common system (pl. 13, *D*, *c*, *d*, *e*, *f*).

In some cases multinucleate spores arise from multiple fusions, but it cannot be taken for granted that all do. Plate 12, *G*, *c*, shows a regular binucleate basal cell. By an irregularity in a later division, one spore (*a*) received 3 nuclei and its intercalary cell received 1 nucleus. On the other hand, there may be multinucleate cells among the basal cells (pl. 12, *C*, *a*) where there is no evidence of any fusion. The later history of such a cell is not known.

The aecium, small at first (pl. 11, *A*, *c*, *d*), spreads by marginal growth (pl. 11, *D*, *c*, *f*, *h*, *j*). As a result of this, a mature aecium shows all gradations, from the youngest stages in the marginal growth to spore chains at the center. Plate 12, *A*, shows a detail from the margin of an aecium with buffer cells arising at *a*, and spore formation in progress at *b*. In the portion of a section shown in plate 13, *E*, the range is from prefusion stages on the left to young spore chains on the right. In this small part of a single section there is evidence of six unmistakable fusions (pl. 13, *E*, *c*, *e*, *f*, *g*, *h*, *i*).

DISCUSSION

The germination of the teliospores to form the promycelia is accompanied by the reduction divisions, and the sporidia produced by the promycelia mark the beginning of the haploid, gametophytic, or unisexual generation. Some, presumably half, of these sporidia are male and half are female, or, to use the other terminology, some are (−) and some (+) in sex. As pointed out by Craigie (*17*, *18*, *19*, *20*), a (+) sporidium grows into a (+) mycelium, which bears (+) pycnia (spermogonia) and (+) pycniospores (spermatia), whereas a sporidium of the opposite or (−) sex grows into a (−) mycelium, which bears (−) pycnia with (−) pycniospores. In other words, if, as a result of the reduction divisions, the original nucleus of a sporidium has the make-up that determines maleness, all the haploid growth from that sporidium and all the numerous descendants of that sporidial nucleus will be male. Spermatia, then, are of both sexes. They are male if borne on a male mycelium, and female if borne on a female mycelium.

In general, the haploid generation of rusts is characterized by uninucleate cells. It is of special interest, therefore, that in flax rust

the nuclear content of the haploid cells is highly irregular. A cell of the vegetative mycelium may contain from 1 to 3 or even 4 nuclei, the majority being binucleate. All the nuclei of a monosporidial mycelium, however, are the direct descendants of the original nucleus in the sporidium, and they bear its sex potentiality. It is a unisexual mycelium and the multinucleate condition of its cells is due to an omission of the formation of septa following nuclear divisions. In the reproductive areas, where regularity in nuclear content is more important, the essentially haploid nature of this mycelium is revealed, for here nearly all of the cells are uninucleate.

Many of the vegetative mycelial cells of flax rust are binucleate, but they are not equivalent to binucleate cells in the sporophyte generation of rusts where one of the two nuclei is male and the other female.

Colley (*16*) reports that in *Cronartium ribicola* Fisch., another rust of the Melampsoraceae, the haustoria produced by gametophytic mycelium are regularly uninucleate and those in sporophytic mycelium are binucleate. In *Melampsora lini*, on the contrary, gametophytic haustoria are as aberrant as the mycelium that produces them and may have from 1 to 7 nuclei.

In the heterothallic species of *Puccinia* investigated cytologically, the haploid mycelium is composed of regularly uninucleate cells and the sporophyte originates at the points of entrance of spermatial nuclei into receptive hyphae, either between epidermal cells (*4*, *5*) or at spermogonia (*3*, *21*). Here it is possible to trace the spread of the bisexual or sporophytic mycelium by noting the number of nuclei per cell. In *Melampsora*, on the contrary, where the unisexual mycelium is incompletely septate, the whole picture is changed, for mycelial cells with more than one nucleus are none the less essentially gametophytic in character.

An interesting parallel can be drawn between the aberrant haploid mycelium of *Melampsora lini* and the equally aberrant diploid mycelium of *Puccinia glumarum* (Schm.) Eriks. and Henn. In the uredial generation of *P. glumarum* (*2*) the vegetative mycelium consists of large multinucleate cells, but in reproductive areas the sporophytic nature of the mycelium reasserts itself, as additional septa cut the many-nucleated cells up into short, binucleate cells, which then give rise to uredia with regularly binucleate spores. The essentially haploid nature of the mycelium in flax rust and the essentially diploid nature of the mycelium in stripe rust are not lost but merely obscured during the vegetative period of incomplete septation.

In *Melampsora lini* each spermogonium forms beneath a stoma and centers upon it. This is not unique in this group of rusts. Dodge (*24*) studied the short-cycle pine rust, *Gallowaya pinicola* Arth., and found that its "vestigial" spermogonia are similarly located beneath stomata and that the spermatiophores of a spermogonium converge upon its apical stoma.

Flax rust is heterothallic. The sporidium grows into a mycelium which, because it is unisexual, cannot carry out sexual reproduction alone. The isolated, monosporidial infection grows old and dies without developing aecia. Only when (+) and (−) elements are brought together does an aecium develop.

In a minority of cases it is possible that the paired cells that fuse in the young aecium are both derived from mycelium of sporidial origin.

It is probable that where infections are crowded together and by chance two infections of opposite sex overlap, their hyphae cooperate directly in the building of an aecium. Even here it should be borne in mind, however, that when the mycelia of two infections are confluent within the leaf their spermatial exudates on the leaf surface are also mixed and there would be an opportunity for sexual reproduction by means of the spermatia. In the great majority of cases, however, (+) and (−) elements are brought together by the transfer of spermatia from one infection to another. There is ample evidence that spermatia of either sex placed upon the surface of an infection of opposite sex germinate and enter in some fashion, and that 24 hours after the transfer young aecia are forming.

Even with this knowledge that mycelium of spermatial origin exists and with abundant material for study, it has been difficult to learn much about this 24-hour interlude.

It is probable that the transferred spermatia, by chance placed upon the ostiole of a spermogonium, germinate and grow in through the spermogonium, using the liquid of the exudate as food. Early stages of germination are frequently seen. Later stages are indistinguishable from the delicate paraphyses growing outward.

Observations likewise indicate that spermatia smeared along the epidermis covering an infection may enter epidermal cells. In dealing with objects as minute as spermatia, however, it is difficult to be sure. Against this it would be easy to argue with plausibility (1) that the series of changes seen in spermatia on the surface of the leaf are degenerative in character and lead to death, (2) that stages seen in the actual passage through the outer epidermal wall are few, and (3) that the spermatia found inside epidermal cells are transferred there accidentally by the knife in sectioning. The stubborn fact remains, however, that spermatia can enter 8-day infections in which there are no spermogonia and no receptive hyphae.

In a damp chamber the spermatia may germinate, forming a short surface hypha before penetrating the outer epidermal wall. This suggests that under suitable conditions a spermatium might grow in through a stoma, but no such case has been found.

It seems incredible that so small a cell as the spermatium could make its way through the substantial outer wall of the epidermal cell. The penetration of the cuticle would probably be effected by mechanical force, for the work of Blackman and Welsford (*9*), Brown (*13*), and Dey (*23*) has shown that in general the cuticle of plants is not dissolved by the enzymes secreted by fungi. As soon as the cuticle is perforated the spermatium has access to water. This would reduce the main hazard of the little cell, namely, its death by drying.

Flax rust as a whole is rich in wall-dissolving enzymes. Tobler (*51, p. 225*) says, "Es tritt nämlich Hyphengeflecht auch in den Bastfasern auf und es erhalten vorher dickwandige Bastfasern allmählich dünnere Wände, ohne dass zunächst ihr Umfang sich verändert." Later these weakened fibers collapse. They have no commercial value—they are unusable. That the spermatia themselves can secrete wall-dissolving enzymes is evident from the thin place in the epidermal wall beneath a spermatium, even when the latter does not enter.

As to the time required for germination, Hart (*31*) finds that aeciospores of flax rust start to germinate in 45 minutes and urediospores in 1½ hours. This compares well with spermatia that are

found in epidermal cells 1½ hours after being placed on the leaf. So fragile and evanescent a cell as a spermatium would be lost if it could not enter quickly.

In isolated infections no case has been found in which the spermatia produced by an infection reenter it. It is conceivable that spermatia grow only under the stimulus of the presence of an infection of opposite sex. Although nothing is known of the chemical and physiological differences between male and female in the rusts, it is probable that such differences exist. Work on the mucors (*45*, *46*, *47*, *48*, *49*) has shown several chemical differences between (+) and (−) strains. The gametophytic infection of flax rust has a pronounced odor. It may be that the emanations from (+) and (−) infections differ. Any living, growing cell may be presumed capable of chemotropic responses.

The germ tube from the spermatium is very slender and delicate. Twenty-four hours after germination, when the hyphae, male and female, that are to fuse, grow out to the subepidermal region, they are equal in size and similar in appearance. It has not been possible to trace the spermatial hyphae all the way from the germination of the spermatium to fusion in the aecium, but the presumption is that following spermatization each cell fusion in the aecium is between a hypha of sporidial and a hypha of spermatial origin.

The existence of cell fusions in the aecium of *Melampsora lini* cannot be questioned. As stated by Fromme (*29*, *p. 122*):

> The abundance of sexual fusions in *Melampsora lini* is most striking. The sexual fusions figured by earlier students have been scattered and disconnected and apparently only occasionally found. In my material I have sections showing practically every pair of gametes in the sorus in some stage of fusion.

The present study of this rust fully confirms Fromme's statements. The cell fusions in the aecium have every appearance of being sexual fusions. This brings up the question of the status of the earlier connections between sporidial and mycelial hyphae (pl. 10, *E*). It is to be wished that more examples had been seen. The evidence available is too limited to be conclusive. If these are true anastomoses between (+) and (−) elements, it would be expected that an exchange of nuclei would follow. But if (+) and (−) nuclei are brought together in the mycelium why is there need for cell fusions in the aecium? It is unlikely that there would be two sexual fusions in the same life cycle.

As suggested earlier, the appearance of these mycelial connections suggests that they may be intimate contacts rather than anastomoses and may serve as a means of extracting food. Spermatia can grow, but no one has ever succeeded in producing an independent infection from rust spermatia. Perhaps a spermatium is capable of only a limited independent growth, and if during this growth it does not succeed in establishing a parasitic relationship with a sporidial mycelium it dies.

Some aecia start at the edge of a spermogonium and others at a considerable distance from spermogonia. It would be interesting to know whether the location of the aecium is decided by the point at which spermatia enter. If spermatia entered only at spermogonia, a considerable distance would need to be traversed to the location of outlying aecia; but if spermatia may enter at any point in the epidermis

covering the infection and if the aecium starts at that same point, the minimum of growth of the spermatial component will be required.

At present it is not known whether other members of the Melampsoraceae are also heterothallic and, if so, whether they have the same method of fertilization as *Melampsora*. In *Cronartium ribicola* (*16*) and the stem-dwelling peridermiums (*1*) the large spermogonia form quantities of spermatia and are visited by insects. Later, beneath this same spermogonial area of the infection, deep-seated aecia develop in which cell fusions take place between intercalary cells of the paired hyphae. These facts suggest the same mode of fertilization as in *Melampsora*, but further work is needed to prove it.

A comparison of the oidia of the hymenomycetes, the microconidia of the ascomycetes, and the spermatia of the rusts is illuminating.

In some rusts spermatia of one sex fuse directly with receptive hyphae of another to start the sporophyte generation (*3*, *4*, *5*, *6*, *21*). In *Melampsora* spermatia first grow into mycelium, which then fuses with mycelium of opposite sex.

In the hymenomycetes, *Coprinus lagopus* Fr. produces oidia which function in sexual reproduction. Brodie (*11*, *p. 343*) says:

> The oidia of *C. lagopus* very closely resemble the pycniospores of the Uredineae in their early appearance on haploid mycelia, in their being immersed in a fluid which is attractive to flies, in their transportation by flies from one mycelium to another, and in their ability to bring about the diploidization of mycelia of basidiosporous origin and of opposite sex.

In the ascomycetes, microconidia play a corresponding role. Dodge (*26*) finds that in *Neurospora* microconidia are borne on mycelia of either sex and carry on the sex of the mycelium that bears them. Microconidia of either sex may be used to "spermatize" a mycelium of the other sex. Microconidia of both sexes can also grow into mycelia that can then be mated with each other.

Drayton (*28*) finds that *Sclerotinia gladioli* Massey is heterothallic. Reproduction takes place by means of microconidia and receptive bodies (columnar structures 1 mm in height, covered with short protruding hyphae). The microconidia cannot grow into mycelium. They become effective only when placed upon the receptive bodies of a mycelium of opposite sex.

Dowding (*27*), working on yet another heterothallic ascomycete, *Ascobolus stercorarius* (Bull.) Schröt., finds that haploid mycelia of either sex produce oidia, which are transferred by mites and flies. An oidium grows into mycelium of the same sex as the mycelium that bore it. Only when it makes contact with mycelium of opposite sex does diploidization result.

The similarity of the role of microconidia, oidia, and spermatia is obvious, and each new addition to the literature makes the parallel more striking. These minute cells, whether microconidia, oidia, or spermatia, are borne on unisexual mycelia of either sex and a given microconidium or oidium or spermatium carries on the same sex as the mycelium that produced it. In some cases such a cell functions only if placed directly on a receptive organ of a mycelium of opposite sex. In others, it may first grow into a mycelium any cell of which can mate with a mycelium of opposite sex. Moreover, in *Coprinus* male and female mycelia, both of oidial origin, can mate with each other. In *Neurospora* male and female mycelia grown from microconidia can mate with each other. It is now known that mycelium

of spermatial origin exists in rusts, but no case is yet known of a mating between (+) and (−) mycelia, both of which arise from spermatia.

The three groups of fungi closely parallel one another in another character. Buller (*15*) found that a diploid mycelium of *Coprinus lagopus* can anastomose with a haploid mycelium and contribute to it nuclei of the sex it lacks, thus diploidizing the haploid mycelium. Brown (*12*) succeeded in diploidizing the haploid mycelium of *Puccinia helianthi* Schw. by using uredial (bisexual) mycelium. Dodge (*25*) obtained hybrids from a cross between a unisexual and a bisexual race of *Neurospora*.

This informal contribution of nuclei from a diploid to a haploid mycelium to start its bisexual generation is now known in a hymenomycete, an ascomycete, and a rust, and may well be found to play an important role in all of these groups of higher fungi. The advantage of such plasticity in reproduction is obvious enough. It is sexual reproduction if by that we mean the change from a unisexual mycelium to a bisexual one, but it involves no "sex organs" or "sex cells." Perhaps the importance of specific sex organs in sexual reproduction in the higher fungi has been overemphasized. A survey of recent literature indicates that for sexual reproduction in higher fungi it is essential only that a male and a female nucleus be brought together in one cell under conditions favorable for further growth, no matter in what fashion this is achieved.

SUMMARY

Flax rust (*Melampsora lini*) is an autoecious, long-cycle rust.

The sporidium germinates and enters the epidermal cell, forming there a primary hypha of several uninucleate cells. This hypha branches and gives rise to intercellular haploid mycelium the cells of which are 1- to 4-nucleate. Haustoria contain from 1 to 7 nuclei.

Spermogonia form first at the upper surface and later at the lower. Hyphae, here predominantly uninucleate, mass between the epidermis and the palisade. Growth in a spermogonium centers on a stoma. This apical stoma is used as an ostiole. Paraphyses grow out through it and spermatia (uninucleate) ooze from it. Later, the epidermis over a spermogonium is sloughed off.

Flax rust is heterothallic. Isolated monosporidial infections grow old and die without producing aecia. The transference of spermatia of one sex to a mycelium of opposite sex leads to the formation of aecia in infections 8 to 20 days of age—rarely after that. In the material studied there were indications that only two sexual groups were present.

Spermatia placed upon the surface of an infection enter, probably growing in through spermogonia, perhaps also entering through epidermal cells, and then grow into intercellular mycelium the hyphae of which are at first very fine, but later of ordinary appearance. There are indications that spermatial hyphae obtain food from the sporidial hyphae.

Soon afterward hyphae (predominantly uninucleate) of both sexes grow out to the epidermis (either upper or lower), cut off 1 to 4 layers of buffer cells, and then fuse by pairs to form the "two-legged cells." The majority of these fusion cells are binucleate, but deviations and irregularities are common. Each of the fusion cells cuts off terminal binucleate spore initials, each of which divides again into

the definitive spore and the intercalary cell. The continued repetition of this gives rise to a chain of spores. About 4 days after spermatia are placed on an infection, its open aecia are shedding spores.

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INFLUENCE OF SOIL MOISTURE ON LONGEVITY OF COTTON ROOT-ROT SCLEROTIA [1]

By C. J. KING, *agronomist*, and E. D. EATON, *junior pathologist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

The fungus *Phymatotrichum omnivorum* (Shear) Duggar, the cause of cotton root rot, has three known stages in its life history, namely, (*1*) a vegetative or *Ozonium* stage (*6*),[3] (*2*) a conidial or *Phymatotrichum* stage (*1*), and (*3*) a sclerotial stage (*4*).

Several investigators have shown that the sclerotia, or hold-over bodies, are usually present in the soil wherever the disease is found, and play an important part in the persistence and overwintering of the fungus. Dana (*2*) found viable sclerotia that had survived a fallow treatment of more than 2 years, and King and Hope (*3*) found that a large proportion of sclerotia were viable after being maintained for 2½ years in soil cultures in the laboratory. Neal (*2*), King, Loomis, and Hope (*5*), and Dana (*2*) reported that the viability of sclerotia of ordinary size was destroyed when they were exposed to drying for a very short time. King, Loomis, and Hope (*5*) found that a large proportion of sclerotia were viable after immersion for 120 days in water, and Taubenhaus, Ezekiel, and Lusk (*7*) found that flooding field plots for 120 days failed to eradicate the disease, although they observed that the vegetative stage of the fungus was no longer viable after submergence in saturated soil for 3 days.

Additional information concerning the influence of soil moisture on the germinating powers of sclerotia seemed desirable. An experiment was conducted, therefore, in which the sclerotia were buried for varying periods in soils maintained at different definite moisture contents.

EXPERIMENTAL PROCEDURE

Fifteen unglazed flowerpots, each with a capacity of 10 pounds of dry soil, and 15-quart tin cans, were used as containers for the moisture-control experiment. The drainage hole at the bottom of each pot was plugged, and the entire outside surface was covered with paraffin to reduce evaporation. Covers made of thermogen paper were used to prevent excessive loss of moisture from the surface.

The soil used was an unsterilized Gila fine sand, which at saturation contains about 27 percent of moisture on a dry-weight basis. Before placing the soil in the pots and cans it was air-dried, sifted to remove plant roots, and thoroughly mixed with measured quantities of water to make up to the desired proportions.

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[2] The writers are indebted to Claude Hope, junior horticulturist, and Orlan Parker, assistant scientific aid, Division of Cotton and Other Fiber Crops and Diseases, for assistance in conducting the experiments.

[3] Reference is made by number (italic) to Literature Cited, p. 798.

One series of 3 cans was maintained without addition of water, and another series of 3 cans was maintained at 5-percent moisture. Three other series for each moisture content, of 5 pots and 3 cans each, were made up to contain 5, 10, 25, and 28 percent of moisture. The moisture was maintained approximately at these percentages by weighing the pots at frequent intervals throughout the period of the test, and adding water to replace that lost by evaporation.

The sclerotia for the experiment were obtained from an infested alfalfa field a short time before the experiment was begun. Nearly all of them were reddish brown in color and firm in texture, which

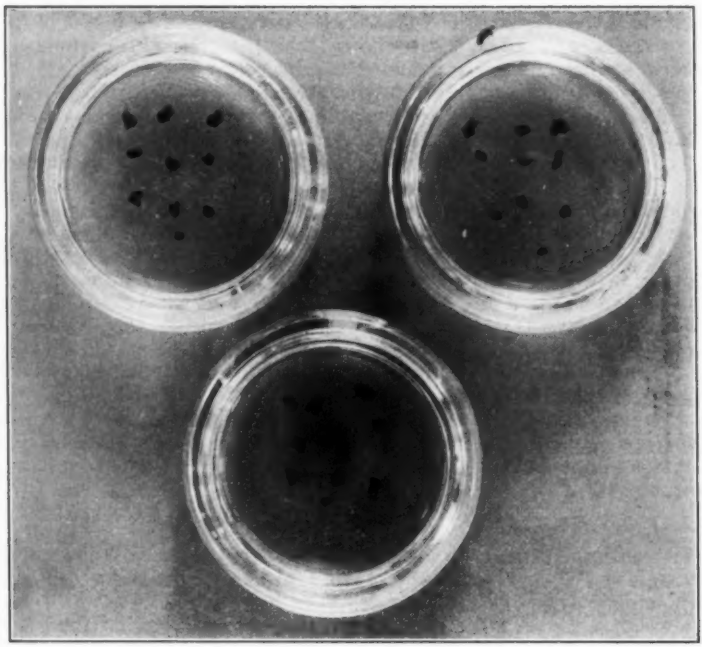

FIGURE 1.—Representative lots of root-rot sclerotia of the sizes and type used in viability studies under different soil-moisture conditions. ×3/5.

suggested that they may have developed during the previous season. A preliminary test showed that about 80 percent of them were viable, although some were unusually slow in germinating. It was not possible to obtain an adequate number of whitish newly developed sclerotia at this period of the year, and the type produced in cultures was not considered desirable. They were arranged in representative lots of 10, consisting of 3 large, 6 medium, and 1 small specimen (fig. 1). Each lot of 10 was placed in a small bag made of cheesecloth with a small quantity of soil about them in the bag to simulate natural conditions of soil contact. Five of the bags thus prepared were buried in each pot and 2 in each can at a depth of 4 to 6 inches, and the soil was compacted about them. The containers were then

covered and stored in a basement, where the temperatures fluctuated only 2° to 4° (C.) within 24 hours.

The containers were prepared on May 7, 1932, and once a month thereafter an effort was made to recover representative samples of at least 10 sclerotia from each moisture series. These were tested for germination by placing them on dark-colored paper in moist chambers and incubating at approximately 25° C. for 10 days.

RESULTS

At the end of the first month only 20 percent of the sclerotia recovered from the dry soil were viable, and at the end of the second month 15 percent. After 1 month 89 percent of those tested in the 5-percent moisture series were viable, and after 2 months 33 percent. All sclerotia in these 2 series were dead at the end of 3 months.

After the experiment had been in progress for 4 months it became evident that some sclerotia were germinating in place in the soil. This was first observed in the pots maintained at 25-percent moisture. In October it was noted that some sclerotia in all 5 pots of the 25-percent moisture series had germinated in place, and an extensive mycelium had formed in byssoid tufts or thin mats on the outside of some of the pots, indicating that the hyphae had penetrated the clay walls and reached the surface where the paraffin covering was thin or had accidentally been rubbed off. A mycelial growth was also observed on the underside of the thermogen-paper covers, spread out in characteristic anastomosing fashion. On the same date patches of mycelium were noted on 2 of the 5 pots containing 28-percent moisture and also on their covers. Only 1 of the pots maintained at 10-percent moisture showed this emergence of the mycelium, but about half of the sclerotia in this series had germinated by November.

Most of the cheesecloth bags had become badly decomposed by December, and many of the sclerotia having germinated in place, it became increasingly difficult to recover the desired numbers for making the monthly viability tests. After this date it was hardly practicable to recover the same number each time, those that had decomposed to the condition of husks not being considered.

Before any container was discarded the soil was sifted and washed in an effort to recover surviving sclerotia that had been overlooked in previous sampling. Records were made of the percentage of recovered sclerotia that germinated, these figures not including sclerotia that probably germinated in place and disintegrated.

It is recognized that the small number of sclerotia recovered for some dates and the necessary omission of sclerotial husks and fragments from consideration may introduce elements of doubt. It is believed, however, that the data may serve as an index of the proportions of sclerotia that may retain or lose their viability and of those that may germinate in place and disintegrate under similar soil conditions in nature.

The number of sclerotia tested and the percentage that were viable in the different series at the sampling dates for the period June 7, 1932, to May 7, 1933, are given in table 1. The percentages of viable sclerotia at each monthly sampling are also shown graphically in figure 2.

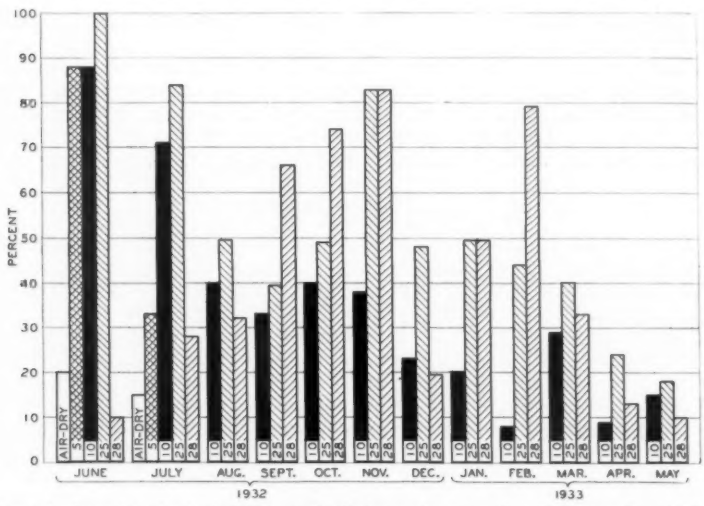

FIGURE 2.—Percentage of root-rot sclerotia germinating in samples recovered monthly from soils maintained at different moisture contents.

TABLE 1.—*Percentage of root-rot sclerotia germinating from samples recovered monthly from pots and cans of soil maintained at definite moisture contents*

Month	Date	Air-dry		Moisture 5 percent		Moisture 10 percent		Moisture 25 percent		Moisture 28 percent	
		Sclerotia recovered	Germinated	Sclerotia recovered	Germinated	Sclerotia recovered	Germinated	Sclerotia recovered	Germinated	Sclerotia recovered	Germinated
		No.	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*	*No.*	*Pct.*
1	June 1932	10	20	9	89	9	89	7	100	10	10
2	July 1932	26	15	18	33	25	72	20	85	25	28
3	August 1932	20	0	25	0	20	40	20	50	19	32
4	September 1932					12	33	10	40	12	67
5	October 1932					15	40	22	50	20	75
6	November 1932					16	38	18	83	12	83
7	December 1932					18	23	38	48	15	20
8	January 1933					10	20	14	50	12	50
9	February 1933					15	8	9	45	14	80
10	March 1933					10	30	10	40	6	33
11	April 1933					10	10	12	25	8	13
12	May 1933					20	15	22	18	20	10
Total		56	.....	52	.....	180	.....	202	.....	173	.....
Number not recovered, probably decomposed		.....	.....	.....	.....	130	.....	108	.....	137	.....

The data indicate that root-rot sclerotia do not survive for a great length of time in soils of low moisture content. In the soil maintained at 10-percent moisture a considerable proportion of the sclerotia remained viable during the first 10 months. After the first 2 months, however, many of those that gave no growth in moist chambers had already germinated in the pots and cans. In the containers maintained at 25-percent moisture the proportion viable at each test was

greater than that in the 10-percent series, but with these also the percentage of viable sclerotia decreased rapidly after the ninth month.

The germinating behavior of the sclerotia in the series maintained at a little above saturation (28 percent) was somewhat erratic. Only a few of the sclerotia recovered during the first 3 months of the test were viable. However, from the fourth to the tenth month the proportion of viable sclerotia in this series was usually higher than in any other. The low viability during the early months of the test is difficult to explain. Since the soil was not sterilized, it is possible that sclerotia in some pots of this series may have been parasitized by bacteria or other organisms and their viability destroyed.

Sclerotia can be preserved in flasks of water for several months. It has been observed, however, that specimens lying near the surface of the water may germinate and produce hyphae and strands which extend through the water and develop further growth on the surface.

In the soil tests viabilty was better preserved at the higher moisture percentages, to this extent approaching results with water submergence. The spontaneous germination which occurred in soils maintained near the saturation point, and the mycelial growth which developed on the outside of the pots suggest a further parallel to the reaction of surface-lying sclerotia in water-preserved samples. Results in both cases suggest that only a small amount of air is needed for germination when temperature conditions are favorable.

In previously reported studies on the germination of sclerotia there is no record of direct evidence that sclerotia germinate in the place of origin without artificial stimulation. Their ability to do this under natural conditions has been assumed. It is known, however, that sclerotia may remain in undisturbed cultures for years without activity all that is required to induce immediate germination being transfer to moist-chamber conditions. McLean (*2*) grew seedlings of pea, bean, and cotton in soil cultures which contained the active fungus, and no infection on the roots was observed, although they penetrated masses of sclerotia. The writers likewise observed that sclerotia were not activated by roots of susceptible seedlings, although infection in the form of small lesions did occur occasionally from growing hyphae.

In addition to the observations made on spontaneous germination in pots of soil maintained at different percentages of moisture, the same occurrence was noted in stock soil cultures of the fungus in Mason jars kept in the laboratory. One jar inoculated on May 13, 1932, was examined on August 8 and found to contain abundant mature sclerotia, but no actively growing mycelium. An examination on September 1 revealed the fact that sclerotia at different places in the jar had germinated, forming patches of white mycelium on their surfaces and against the sides of the jar. Some of the sclerotia were removed for observation, and the hyphae extending from their surfaces were examined microscopically and found to be of recent growth. The jar was placed in an incubator at 28° C., and on September 5 the white patches had increased in size. During the next 2 weeks numerous fine strands were formed, but no growth was observed after October 4, although moisture conditions appeared to be favorable.

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SUMMARY

Sclerotia of the root-rot fungus, *Phymatotrichum omnivorum*, were buried in pots and cans of soil maintained at different moisture contents. One series was air-dry and others were kept at 5, 10, 25, and 28 percent. Samples were recovered and tested for germination at monthly intervals for 1 year.

All sclerotia in the dry soil and in that kept at 5-percent moisture were dead at the end of 3 months. Those in the soil at 10-percent moisture showed a gradual decline in viability after the first 2 months, with a small proportion still viable after 12 months.

Viability was somewhat better preserved in soils maintained at 25- and 28-percent moisture, though behavior at 28 percent was somewhat erratic, suggesting that some sclerotia were injured by other organisms.

Spontaneous germination occurred in some of the containers maintained at 10-, 25-, and 28-percent moisture, and in some culture pots the hyphae penetrated the clay sides and the mycelium developed into extensive mats on the outside. Germination and hyphal growth may take place to a limited extent even under saturated-soil conditions where little air is available.

Sclerotia were observed germinating in their place of origin in soil cultures several months old, and growth of the resulting mycelium was maintained for several weeks.

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ABERRATIONS IN THE CHEMICAL COMPOSITION OF PEAS FROM PLANTS AFFECTED WITH ROOT ROT [1]

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INTRODUCTION

In 1932 Horsfall, Kertesz, and Green (*9*) [2] reported some physiological observations and a few chemical analyses of peas from normal and root-rot-affected plants. Since these results showed significant differences in the proportion of dry matter as well as in the constitution of the dry matter itself, they were used as the basis for a further study extending through the 1932 and 1933 seasons. The present paper deals with the progressive chemical changes that take place during the harvest season in peas from diseased vines as compared with those from normal vines growing under similar conditions.

The literature is sprinkled with references to the influence of a fruit disease on the composition of the fruit, but references to the influence of a root disease upon the composition of the fruit are rare. Dungan (*6*) reported briefly that corn root rot reduced the specific gravity of the grain as well as the proportion of total sugars and ether extract. The nitrogen content of the tissue seemed to be increased by the disease. Linford (*15*) found that the dry matter content of pea plants affected with wilt was higher than that of normal plants. Culpepper and Magoon (*4*) studied the effect of artificial root pruning, which might be similar to the effect of root rot, on the chemical composition of sweet-corn kernels. They found that total and alcohol-insoluble solids and acid-hydrolyzable higher carbohydrates in sweet-corn grains increased with the extent of root pruning performed at the time of silking. Moreover, "the rate of maturing seemed to be slightly increased."

MATERIAL AND METHODS

SAMPLING TECHNIC

The variety Perfection was used throughout the experiments. The technic employed in collecting infected and healthy plants and sampling them has already been fully discussed (*7*). It may be described briefly as follows: In 1931, peas (*Pisum sativum* L.) were obtained from yellowed vines and from normal-looking vines growing near-by in the same field. This conscious selection of diseased and healthy samples gave large differences in the results. In 1932, a plot of normal soil was artificially infested with "pea-sick" soil. Thus normal and diseased plants could be grown on contiguous plots under otherwise identical conditions. The 1933 samples came from the same plots as the 1932 samples.

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[2] Reference is made by number (italic) to Literature Cited, p. 813.

In all of these studies samples for analysis were drawn from the population at intervals during the season. This method of obtaining samples gives data that can be arranged in trend curves which show their significance at a glance without resort to statistical analysis.

In all years whole-plant samples of the two lots were broken at the ground line before 8 a.m. and taken to the laboratory. Generally, not more than 3 hours elapsed from the time the plants were pulled until all determinations on the fresh peas were finished. Immediately after hand-shelling, each sample was passed through a set of hand sieves corresponding to those used by canners (*7*), and the weight of each fraction was recorded. Samples for analysis were taken from each of the sieve sizes in all cases in which sufficient peas were available. Data on the various sieve sizes are valuable for certain purposes, particularly to canners who are interested in certain sized peas, but they are somewhat unsatisfactory for giving a picture of the situation in a handful of unsifted peas. This difficulty was obviated by taking a weighted average of the determinations for the various sizes. This figure is comparable to average sieve size as already described (*7*, *p.* 7). It is a simple arithmetical calculation involving the multiplication of the number for the chemical constituent in question by the percentage by weight of peas in that size. These numbers for all sizes are then summed and divided by the total percentage of peas in the sizes used. This weighted average determination undoubtedly represents more nearly the true condition in a handful of unsifted peas as obtained from the field than a single determination, as it is essentially a method of replication. It is, however, more than a series of replicated determinations on the original unsifted sample as it gives an idea of the situation in the various components of the original as well.

CHEMICAL ANALYSES

For the determination of the dry matter in the peas, 25- or 50-g samples were dried at 95° to 98° C. for 2 days, after which several controls failed to show a further decrease in weight. Ash, nitrogen, and crude fiber were determined by the official methods (*1*). The determinations of the two fractions of nitrogen were made by extracting the ground peas with hot 80-percent alcohol until the extract failed to give a reaction for sugars. In the extract the sugars were determined by the procedure described earlier (*14*), Bertrand's method and the recalculated tables (*11*) being used. Starch determinations were performed on the alcohol-insoluble residue, using takadiastase followed by acid hydrolysis (*12*). The figures for total carbohydrates in 1931 were obtained by direct acid-hydrolysis of the dried peas by the method given by Boswell (*3*). The figures for total carbohydrates in the peas of 1932 were obtained by subtracting the percentage of nitrogen $\times 6.25$ from the percentage of alcohol-insoluble residue and adding the percentage of total sugars; consequently they include crude fiber.

ORGANISMS ISOLATED

Isolations from diseased roots on the experimental plants show that *Pythium* spp., favored by a wet May, were the primary invaders in the roots in 1931 and 1933 and that *Rhizoctonia solani* Kühn, favored by a dry May, was the primary invader in 1932 (*7*, *8*). Both organisms were found in the roots during each season, so that the symptom

complex called root rot cannot be ascribed to the action of a single organism. Technically the data might have been more widely usable if they had been taken from plants inoculated with single organisms, but in other respects the data are more applicable because they deal with the actual situation as it exists in the field and as it confronts the canner.

If *Aphanomyces euteiches* Drechs. occurred it was not important in in the etiology of the complex. The plants were considerably injured by aphids in 1933, and the onset of the disease was much earlier in that year than in 1932, so that the plants were infected for a longer period.

WEATHER CONDITIONS

In all 3 years June was dry, with no appreciable rainfall until 2 or 3 days after sampling began. A drought-breaking rain fell on July 3 in 1931, on July 1 in 1932, and on June 30 in 1933. In table 1 the rainfall is shown for the three seasons in which the samples were taken. No further rain fell during the harvest season in 1931, so that the peas were very hard by July 6. The same was essentially true in 1933, but in 1932 the harvest season after July 1 was moist. For commercial use the peas would have been ready for a fancy pack on July 2 in 1931, on July 5 in 1932, and on July 1 in 1933.

TABLE 1.—*Daily precipitation (inches) during the pea harvest season at Geneva, N.Y., in 1931, 1932, and 1933*

Year	June				July														
	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1931							0.99	0.04		0.03	0.04	(1)	(1)	0.77	0.07	0.01		0.20	
1932	0.33			(1)	2.11	0.05	.04	.95			.18	0.44	(1)	(1)	.55		(1)	.14	
1933			(1)	0.37	.01	(1)	.30					.01	0.06	(1)					

[1] Trace.

PRESENTATION OF RESULTS

DRY MATTER

The main chemical effect of the root-rot complex on the pea plant is an increased dry-matter content resulting from a curtailment of the power of water absorption through the diseased roots. Consequently, peas from plants affected with root rot contain less water than peas from healthy plants. In table 2 and figure 1 the results of the dry-matter determinations on Perfection peas for 1931, 1932, and 1933 are presented. This table also contains the percentages by weight of peas in certain sizes on the different sampling dates. These figures have been used to calculate the average sieve size of the peas (7), which was used as a basis for calculating the average content of the different constituents, as described on page 799.

Since in 1931 the diseased peas were taken from spots showing symptoms of root rot, while the control samples were obtained from an adjacent spot where relatively disease-free plants were growing, the differences between the two samples were much larger than in later years when the samples were obtained from contiguous plots one of which was contaminated with "pea-sick" soil. The effects of the abnormally dry season of 1933 are apparent in the dry-matter

curves, which show that the dry-matter content of the peas at the very beginning of the season (June 30) was higher than at the end of

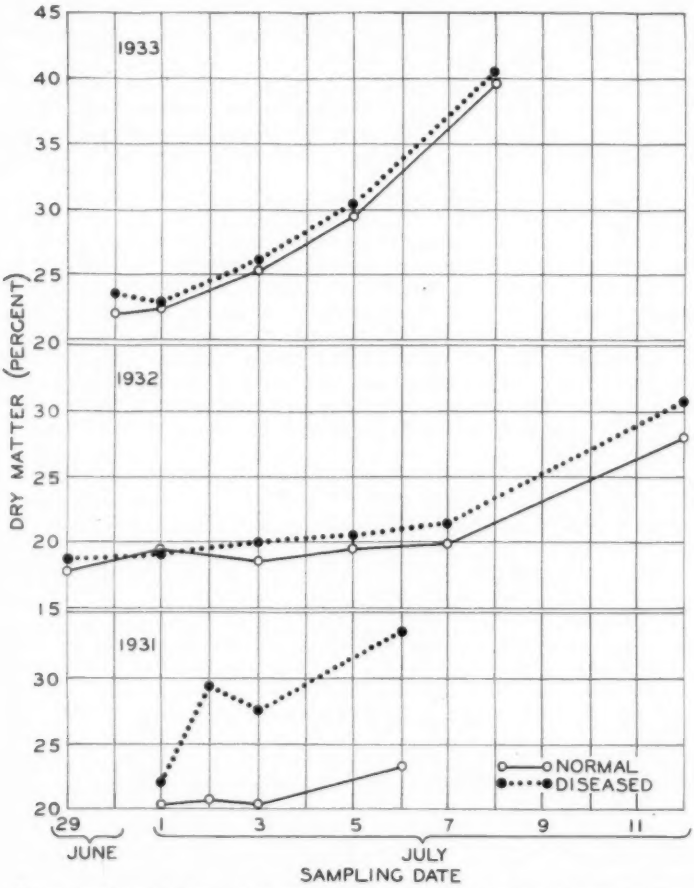

FIGURE 1.—Dry matter (percent) of normal and diseased Perfection peas in 1931, 1932, and 1933.

the canning season (July 7) in 1932. The quality of all peas harvested in 1933 was poor even when the peas were in "prime" condition.

TABLE 2.—*Average sieve size, distribution of sizes, and dry matter content*[1] *of normal and diseased Perfection peas in 1931, 1932, and 1933*

Date of harvest	Normal peas			Diseased peas		
	Sieve size	Distribution of sizes, by weight	Dry matter	Sieve size	Distribution of sizes, by weight	Dry matter
1931		*Percent*	*Percent*		*Percent*	*Percent*
July 1	2	21.3	18.91	.....	22.2	20.89
	3	23.8	19.78	.....	37.5	22.07
	4	27.2	21.39	.....	26.2	23.35
	5	9.7	21.78	.....	6.1	23.63
Average		2.94	.....	20.33	3.02	.....	22.25
July 2	2	18.7	18.91	.....	26.9	30.79
	3	28.6	20.14	.....	19.2	29.81
	4	40.3	21.37	.....	43.9	28.85
	5	10.5	23.34	.....	.....	.....
Average		3.40	.....	20.75	3.31	.....	29.64
July 3	2	4.5	18.68	.....	.....	.....
	3	28.9	19.80	.....	26.5	28.70
	4	41.0	20.71	.....	45.4	27.20
	5	21.8	22.11	.....	23.3	28.21
Average		3.75	.....	20.66	3.92	.....	27.87
July 6	3	12.2	21.32	.....	25.1	35.26
	4	47.4	23.32	.....	38.7	32.84
	5	29.5	24.61	.....	19.3	33.11
Average		4.24	.....	23.46	3.62	.....	33.62
1932						
June 29	2	29.60	17.06	.....	33.30	17.93
	3	18.08	18.01	.....	27.70	18.69
	4	12.16	18.92	.....	13.80	19.71
	5	3.54	19.57	.....	2.80	20.80
Average		2.06	.....	17.80	2.41	.....	18.63
July 1	1	11.15	16.49	.....	15.30	16.22
	2	17.45	17.23	.....	19.10	17.07
	3	27.65	17.66	.....	20.90	18.85
	4	26.45	18.79	.....	20.40	19.86
	5	15.35	20.10	.....	21.60	20.45
	6	1.95	20.73	.....	2.70	22.35
Average		3.23	.....	19.53	3.23	.....	19.21
July 3	1	4.85	16.37	.....	4.30	.....
	2	7.45	17.12	.....	8.55	17.14
	3	13.10	17.29	.....	10.20	18.17
	4	27.10	18.15	.....	23.60	19.54
	5	39.10	19.59	.....	40.80	20.55
	6	8.40	21.00	.....	12.55	21.39
Average		4.08	.....	18.37	4.31	.....	19.85
July 5	2	2.05	16.18	.....	3.87	17.17
	3	9.92	17.37	.....	5.40	17.93
	4	14.40	18.90	.....	14.00	19.13
	5	36.80	18.90	.....	34.20	20.45
	6	33.50	21.13	.....	39.60	21.36
Average		4.80	.....	19.46	4.92	.....	20.36
July 7	2	2.10	16.21	.....	1.72	17.99
	3	4.20	17.36	.....	2.86	17.22
	4	9.30	19.06	.....	6.90	18.84
	5	27.50	18.70	.....	27.60	21.06
	6	55.00	21.05	.....	59.60	22.77
Average		5.23	.....	19.94	5.37	.....	21.75

[1] In this table only the percentages of sizes and dry-matter content of the samples used for chemical work are given. For further details see (*9*) for the 1931 and (7) for the 1932 samples.

TABLE 2.—*Average sieve size, distribution of sizes, and dry matter content of normal and diseased Perfection peas in 1931, 1932, and 1933*—Continued

Date of harvest	Normal peas			Diseased peas		
	Sieve size	Distribution of sizes, by weight	Dry matter	Sieve size	Distribution of sizes, by weight	Dry matter
		Percent	*Percent*		*Percent*	*Percent*
July 12	3	0.94	23.34		0.95	27.35
	4	5.66	25.01		3.55	29.50
	5	24.85	27.25		27.49	30.80
	6	67.80	28.70		67.63	31.11
Average		5.58	27.92	5.61		30.92
1933						
June 30	1	8.00	19.04		6.35	18.47
	2	12.62	20.01		10.35	20.18
	3	32.30	22.68		29.45	21.33
	4	37.30	22.74		35.86	22.96
	5	8.78	23.89		16.98	23.96
	6				1.01	24.29
Average		2.25	21.95	3.50		23.59
July 1	1	5.83	17.62		4.20	
	2	11.73	20.98		7.00	19.80
	3	36.80	22.41		21.60	21.74
	4	31.52	23.41		40.50	23.16
	5	13.60	23.94		23.65	23.74
	6	.52			3.05	24.77
Average		3.37	22.49	3.81		22.79
July 3	1	2.48	22.31		1.84	
	2	5.48	23.12		2.35	23.27
	3	21.10	24.50		11.52	23.86
	4	48.62	25.25		46.13	25.46
	5	20.00	26.85		36.22	26.24
	6	2.32	27.04		3.14	27.25
Average		3.85	25.26	4.26		25.88
July 5	1	2.34	27.57		2.80	26.05
	2	5.45	28.35		5.40	30.23
	3	17.64	29.03		16.20	30.77
	4	43.33	29.87		37.10	29.70
	5	27.56	29.76		33.20	31.24
	6	3.68	30.31		5.30	30.31
Average		4.02	29.57	4.08		30.34
July 8	1	9.98	36.15		9.31	34.85
	2	14.22	35.53		13.35	35.40
	3	22.45	36.70		20.19	35.87
	4	35.15	39.75		35.45	40.19
	5	14.65	47.05		18.85	50.02
	6	3.55	53.43		2.85	58.28
Average		3.41	39.66	3.51		40.54

ASH

The results of the ash determinations obtained in the three seasons are presented in figure 2. In 1931 the ash content of the dry matter in the peas from root-rot-affected plants was lower than that in peas from healthy plants. When the results are calculated on the basis of fresh peas, the ash content of the diseased peas is higher because the diseased peas had a higher dry-matter content than the normal peas.

In 1932 and 1933 the ash content of the dry matter was again lower in the diseased peas. The moist season of 1932 gave only slight differences in the dry-matter content of the two lots of peas; accordingly the ash content of diseased peas calculated on the fresh-material basis

was the same as, or a trifle lower than, that in the normal ones. In 1933, however, when the dry-matter content of both lots of peas was much higher than in previous years, the ash content was actually lower in the diseased peas, even on a fresh-material basis.

It was found by Sayre, Willaman, and Kertesz (*17*) that the ash content of normal growing peas increased almost proportionally with the increase in the dry-matter content, whereas it remained about constant or decreased slightly in the dry material itself as the season advanced. The same has been found in the present study in all 3 years, the fresh peas showing a fairly regular increase in ash content except for July 2 in 1931 and July 3 and 5 in 1932. It is believed, as

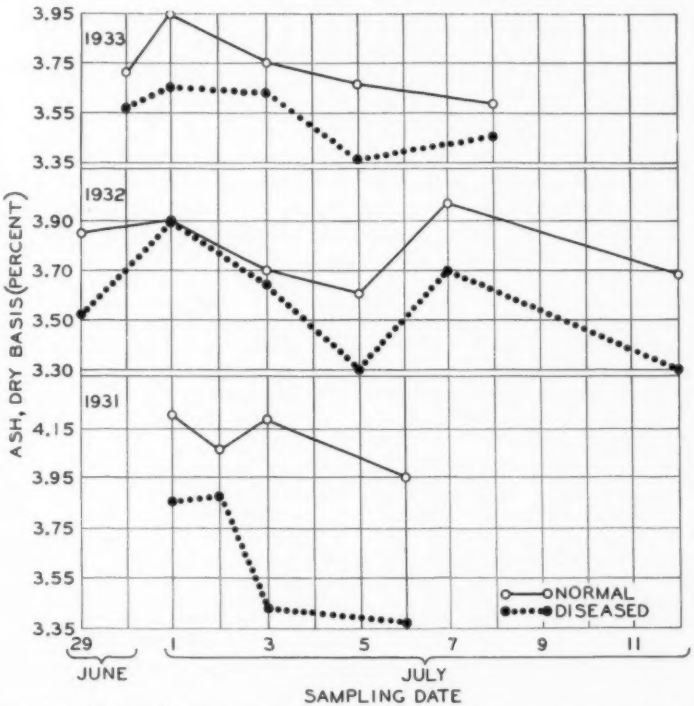

FIGURE 2.—Ash content of the dry matter of normal and diseased Perfection peas in 1931, 1932, and 1933.

suggested by Horsfall, Kertesz, and Green (*9*), that the decrease on these days was due to the heavy rains which fell on July 3, 1931, and on July 3 and 4, 1932. These rains caused the peas to absorb water and dry matter from the vines, which resulted in rapid swelling, as can be seen from the figures for average sieve size given in table 2. Ash uptake lagged behind, the result being a lower ash content in the dry matter for a time.

PROTEIN

The results obtained on the nitrogen content of the peas are presented as percentage of protein (the percentage of total nitrogen multiplied by the factor 6.25). Kertesz and Green (*14*) pointed out

that although a fraction of the nitrogen in peas is present in nitrogenous compounds other than proteins, the actual weight of all nitrogenous compounds is nearer to nitrogen×6.25 than to the weight of nitrogen found.

Horsfall, Kertesz, and Green (*9*) found a lower nitrogen content in the dry matter of diseased Alaska, Advancer, and Perfection peas than in the corresponding normal samples. Because of the large differences in the dry-matter content, this relation was reversed when the results were calculated on a fresh basis, however. The nitrogen

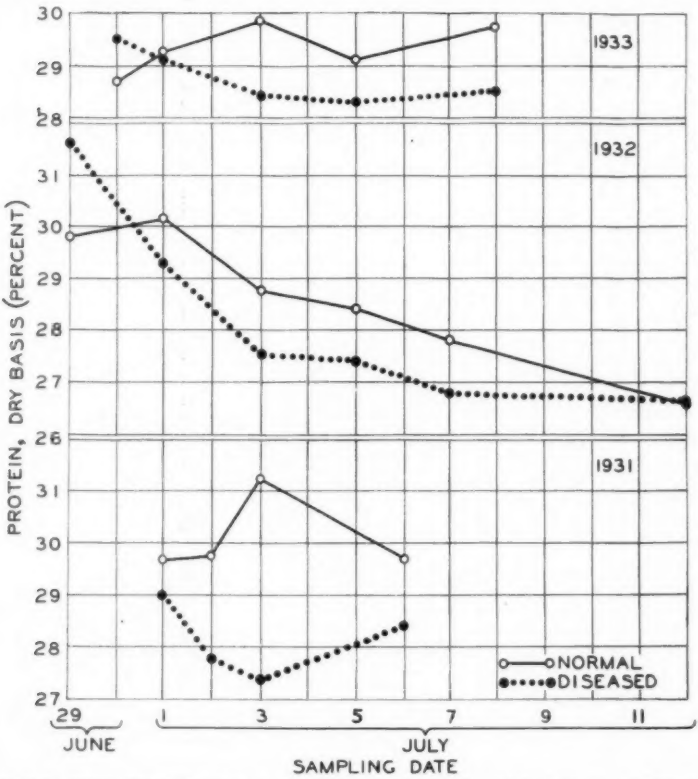

FIGURE 3.—Protein (N×6.25) content, on a dry basis, of normal and diseased Perfection peas in 1931, 1932, and 1933.

determinations on the 1932 and 1933 Perfection peas showed the same relation for nitrogen×6.25 (fig. 3). Except in the first sample in both the 1932 and 1933 seasons, the results confirm the observation that the protein content of the dry matter is lower in the diseased than in the normal peas.

During the growth and ripening of peas, nitrogenous compounds soluble in hot 80-percent alcohol are transformed into insoluble ones (*3*, *10*). Since this transformation is a typical and constant phenomenon in growing peas, the ratio of insoluble nitrogen to soluble

nitrogen has been proposed by Muttelet as an index of ripening (*16*). It was of much interest, therefore, to obtain an insight into the changes in the two fractions of nitrogen in the diseased peas as contrasted with those in normal ones. Table 3 shows the results obtained on the 1932 peas.

The ratios of insoluble to soluble nitrogen were slightly higher for the diseased samples on the first 2 days of sampling, but were lower on July 5. In the latter part of the season 1 ratio was higher and 1 lower. Consequently, the transformation of (mostly) nonprotein nitrogen to protein nitrogen was not significantly more rapid in the diseased peas than in the normal peas.

TABLE 3.—*Weighted average soluble and insoluble nitrogen content of normal and diseased Perfection peas in 1932*

Date of harvest	Normal peas			Diseased peas		
	Soluble N	Insoluble N	Insoluble N / Soluble N	Soluble N	Insoluble N	Insoluble N / Soluble N
	Percent	*Percent*		*Percent*	*Percent*	
July 1	0.158	0.714	4.52	0.153	0.718	4.69
July 3	.149	.716	4.81	.145	.729	5.03
July 5	.108	.776	7.19	.122	.741	6.07
July 7	.080	.814	10.18	.079	.860	10.89
July 12	.038	1.155	30.39	.046	1.277	27.76

CARBOHYDRATES

Since it had been shown (*9*) that the content of both nitrogen and ash in the dry matter of diseased peas is lower than that of normal peas, a search was made for some constituents the proportion of which is higher in the diseased peas and which would thus account for the remainder of the dry matter. Therefore, the 1931 samples were analyzed for total carbohydrates, acid hydrolysis being used to convert the higher carbohydrates into sugars. The results (table 4) show that the content of total carbohydrates is higher in the root-rot-affected peas on both a fresh- and dry-weight basis than in the normal peas. The increase in carbohydrates in the dry matter of diseased peas practically balances the decrease in ash and nitrogen. Additional crude-fiber determinations showed the same general trend as did total carbohydrates, although the differences in this constituent were somewhat inconsistent when expressed on the dry-matter basis.

TABLE 6.—*Weighted average total carbohydrate content (percentage of sugars plus acid-hydrolyzable higher carbohydrates) on fresh and dry basis of normal and diseased Perfection peas, 1931*

Date of harvest	Normal peas		Diseased peas	
	Fresh basis	Dry basis	Fresh basis	Dry basis
	Percent	*Percent*	*Percent*	*Percent*
July 1	9.13	44.89	10.52	47.29
July 2	8.82	42.49	14.24	48.05
July 3	9.18	44.45	15.05	54.00
July 6	10.84	46.19	17.52	52.09

In 1932 an elaborate study of the carbohydrates was made. The season was well suited for such studies since peas of good quality could be obtained and the effect of root rot on the peas in the infested plot was apparent but not severe enough to upset entirely their normal functions, as often happens. The results of the chemical

determinations are presented in table 5. The determinations of reducing sugars are omitted because the content was so low (0.15 to 0.31 percent) and because there appeared to be no difference between the normal and diseased peas in respect to these constituents. To conserve space the figures for higher carbohydrates, including crude fiber, also are omitted from the table since these were obtained by subtracting insoluble nitrogen $\times 6.25$ from the alcohol-insoluble residue. For the same reason, the results are discussed only as daily weighted averages on the basis of the fresh peas.

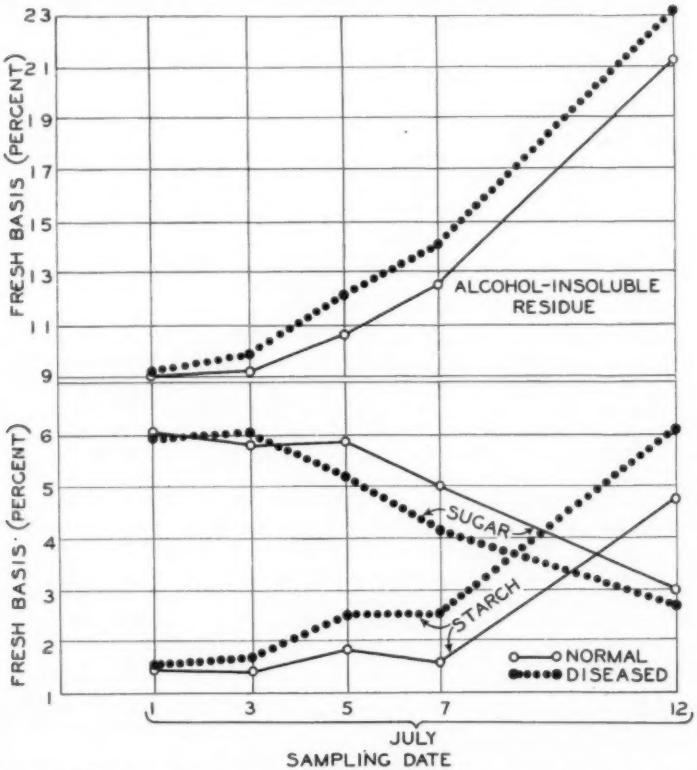

FIGURE 4.—Daily average starch, sugar, and alcohol-insoluble solids content of normal and diseased Perfection peas in 1932.

The total sugar content of the 1932 Perfection peas decreased through the sampling period. The peas were in prime condition for canning on July 5, before the sucrose content of the normal peas has decreased materially. The diseased peas lost only about 11 percent of their average sugar content from July 1 to July 5. After that date the sucrose content of the peas diminished rapidly and was always lower in the peas from diseased plants than in those from normal plants, not only in the daily averages as given, but also when compared size for size. The daily average sugar content of normal and diseased peas is shown in figure 4.

TABLE 5.—*Carbohydrate constituents of fresh normal and diseased Perfection peas, 1932*

Date of harvest	Size no.	Normal peas						Diseased peas					
		Total sugars	Starch	Total carbohydrates	Alcohol-insoluble solids	Higher carbohydrates[1] / sugars	Starch / sugars	Total sugars	Starch	Total carbohydrates	Alcohol-insoluble solids	Higher carbohydrates[1] / sugars	Starch / sugars
		Percent	*Percent*	*Percent*	*Percent*			*Percent*	*Percent*	*Percent*	*Percent*		
July 1	1	4.90	1.47	7.04	6.35			4.91	1.46	5.38	6.65		
	2	5.57	1.22	8.54	7.15			5.49	1.53	7.45	7.27		
	3	5.86	1.51	9.55	8.00			5.77	1.24	9.97	8.71		
	4	6.06	1.31	10.77	9.34			5.90	1.18	11.04	9.91		
	5	5.73	1.10	11.27	10.50			5.69	1.68	11.71	11.12		
Average		6.062	1.449	9.68	9.033	0.60	0.239	5.917	1.468	9.36	9.144	0.61	0.248
July 3	1	5.94	1.28	8.94	6.84			6.74	1.29	9.47	6.77		
	3	6.18	1.09	10.04	7.75			6.18	1.23	9.58	7.40		
	4	5.78	.95	10.04	8.50			6.51	1.63	11.40	9.16		
	5	5.93	1.96	11.73	10.56			5.91	1.90	11.97	10.78		
	6	5.48	1.94	11.80	11.70			5.74	1.68	13.30	12.94		
Average		5.761	1.442	10.80	9.112	.88	.250	6.056	1.667	11.53	9.846	.90	.275
July 5	2							6.41	1.39	9.44	7.20		
	3	6.35	1.37	9.57	7.55			5.85	1.73	10.12	8.21		
	4	6.18	1.75	10.75	8.76			5.98	1.35	10.72	9.35		
	5	5.93	1.80	11.63	10.42			4.97	2.65	12.74	12.58		
	6	5.50	1.96	13.03	12.96			5.17	2.57	13.67	14.00		
Average		5.877	1.856	11.78	10.664	1.00	.316	5.253	2.464	12.55	12.094	1.39	.469
July 7	2							5.58	1.56	11.44	10.48		
	3	5.51	1.11	9.26	8.02			5.05	1.30	9.82	9.13		
	4							5.59	1.40	11.68	10.78		
	5	5.56	1.45	12.66	11.53			4.73	2.04	12.45	12.86		
	6	4.70	1.68	12.92	13.70			3.93	2.82	13.66	15.36		
Average		5.001	1.554	12.66	12.560	1.53	.311	4.187	2.480	13.03	14.016	2.12	.592
July 12	4	3.04	3.48	15.57	18.76								
	5	2.43	5.39	17.34	21.85			2.25	7.41	20.26	25.70		
	6	3.11						2.94	5.74	16.65	21.82		
Average		3.015	4.665	17.11	21.19	4.67	1.547	2.678	6.079	17.69	23.22	5.61	2.270

[1] Including crude fiber.

The sugar content was lower in the diseased samples on all dates, except July 3. During the later growing season the difference in the percentage of sugars in the two lots of peas was almost constant, although this difference expressed as percentage of the total sugar content increased materially as a result of the rapidly decreasing proportion of sugars in the peas. The difference between the sugar content of the normal and the diseased peas is even larger when the results are expressed on the dry-matter basis. On the 5 days when samples were taken, the sugar content of the diseased peas was lower by 0.77, 2.71, 14.57, 23.41, and 14.09 percent, respectively, than the sugar content of the normal peas. These figures well express the progress of the differences between the two lots of peas.

Higher Carbohydrates

The percentage as well as the absolute amount of starch increases in the peas during growth. It is apparent from table 5 and figure 4 that the increase in the starch content was more rapid in the diseased than in the normal peas. In the normal peas there seems to have been only a small variation in the starch content until after July 7, when it increased sharply. In the diseased peas, however, the increase was quite consistent from the second sampling date.

There are other higher carbohydrates which show even more striking differences between the peas from normal and diseased plants. These figures are obtained by subtracting the percentage of soluble nitrogen$\times$6.25 from the percentage of alcohol-insoluble solids. This fraction, which includes starch, dextrines, pentosans, pectins, fiber, and other higher carbohydrates, shows by July 5 a definite difference between the two lots of peas. The differences are much larger than those which the starch content alone could account for. This can be easily proved by subtracting the percentage of starch from the values. The resulting figures still show significant differences in higher carbohydrates other than starch between the normal and diseased peas.

During recent years it has been customary to calculate and apply different "indexes" to the quality of horticultural products. Recently the importance of the proportions of starch and sugar in relation to flavor and quality of sweet corn has been emphasized. Muttelet (*16*) proposed this ratio for determining the quality of peas. In figure 5 the starch-sugar ratios and also the total higher carbohydrate-sugar ratios are presented. As would be expected, the total higher carbohydrate-sugar ratio shows a more rapid increase with ripening and also larger differences between the peas from normal and diseased plants than does the starch-sugar ratio.

It has been shown that in peas sugars decrease, while starch and other higher carbohydrates, fiber, and protein nitrogen increase during growth (*2*, *10*). These constitutents all contribute to the alcohol-insoluble solids of the peas which contain all the "undesirable" constituents of the peas (starch, higher carbohydrates, fiber, insoluble nitrogen, etc.) from the canner's standpoint. This fraction seems to offer a dependable and simple way to evaluate the quality of raw and canned peas (*13*). Figure 4 shows the percentage of alcohol-insoluble residue in the two lots of peas.

The value for alcohol-insoluble solids in the normal peas lagged behind that in the diseased peas by about 1 day, the two curves being parallel. Naturally, the differences in the absolute amount and percentage of alcohol-insoluble solids on the dry-matter basis are much larger than when calculated on the basis of the fresh peas.

DISCUSSION

It is not the purpose of this paper to deal with the effects of root rot on the physiology of the pea plant itself; a preliminary discussion of that point (*9*) has already been presented. Nor is it the purpose to offer a complete explanation of the established differences in the chemical composition of the peas caused by the disease. Knowledge of the mechanism of plant nutrition and assimilation is too meager

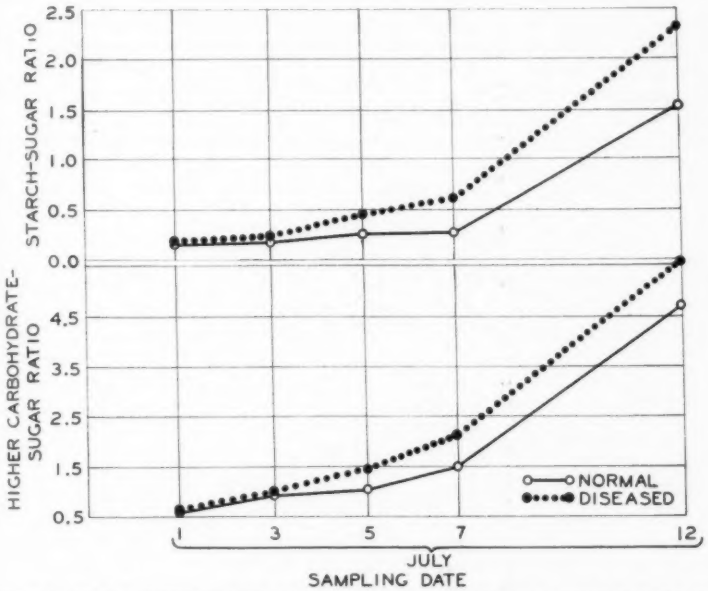

FIGURE 5.—Higher carbohydrate-sugar and starch-sugar ratios of normal and diseased Perfection peas in 1932.

to warrant much speculation regarding the influence of root rot on these processes. Nevertheless, a few points are obvious enough to permit the drawing of some conclusions.

It may be assumed that the primary action of root rot on the pea plant is to curtail the water supply. The obvious results of this are repressed growth or stunting of the diseased plants, the setting of fewer pods per plant, and a lower yield.

As previously stated, the dry-matter content of the diseased peas was higher in all samples taken throughout the three harvest seasons. One might infer that this was due directly to the lowered water intake by the diseased roots. The evidence shows, however, that the higher dry-matter content was due in part, at least, to the pres-

ence of more solid material in the average pea from a diseased plant. The diseased pea grows larger (*6*) and is drier; it must, therefore, contain more solid material than a normal pea.

Since a lower ash and nitrogen content was found in the dry matter of the diseased peas, these two constituents can play no part in an explanation of the higher dry-matter content. Both of these constituents are absorbed through the root system of the plant. When this is damaged by the attacking fungi, the ash and nitrogen uptake is inhibited just as the water intake is. If, as Curtis (*5*) suggests, ash and nitrogen move chiefly through the living tissues of the plant, the destruction of the cortex of the root would be reflected directly in a lessened uptake of these two constituents.

The fact that total carbohydrate content is greater in diseased peas than in normal ones admits of several explanations. The metabolism of the plant may be so upset by the formation of some toxic material in the diseased roots that the synthesis of carbohydrates becomes accelerated. No evidence of this is available in the case of pea root rot, however. It may be that there is a more vigorous translocation of carbohydrates into the ovules, thus leaving relatively less in the plants, or the partial or complete death of the living parts of the root system may prevent it from utilizing as much carbohydrate as otherwise, thus leaving an excess to be moved into the ovules, causing the abnormal enlargement reported (*7*). This hypothesis involves no postulation of a disturbance in the process of translocation.

Of considerable importance is the fact that diseased peas contain relatively less of the lower carbohydrates and relatively more of the higher carbohydrates than normal peas. The excessive enlargement observed (*7*) cannot explain the lower sugar content of the diseased peas, although such an explanation has been suggested. The difference between the average volume of normal and diseased peas on July 5, 7, and 12 was 3.16, 2.18, and 2.29 percent, respectively, while the sugar content of the diseased peas on the same dates was lower by 10.62, 16.28, and 11.18 percent than that of normal peas. Thus, the lower sugar content of the diseased peas cannot be ascribed to a "dilution" of the sugar by an abnormal enlargement.

As already shown (*7*), the peas used for the chemical analyses enlarged until about July 9 in 1932. The normal peas showed no increase in the percentage of starch up to July 7, although naturally the absolute amount of starch per pea increased as the size of the pea increased. Between July 7 and July 12, starch content increased sharply as the peas dried out in approaching the seed stage. In the diseased peas, however, the starch content had already begun to increase by July 5, so that the chemical composition showed signs of approaching the seed stage much earlier than normal.

Carbohydrate changes play an important part in the ripening of green peas. During the early stages of development, the sugar content of peas increases (*2*, *10*). Soon afterward a sharp decrease in the content of total sugars coincident with an increase in higher carbohydrates sets in and continues almost until the peas dry out. Specifically, the lower carbohydrates, like sugars, are changed into starch and other higher carbohydrates. Evidence presented herein indicates that this process proceeds more rapidly in the diseased than in the normal peas. Since polymerization of carbohydrates results in the

production of free water, the physiologically drier conditions existing in the diseased peas would tend to shift the equilibrium in the direction of a smaller water requirement for the system. Thus, the formation of a larger proportion of higher carbohydrates in the peas on diseased plants might be due to a hastening of polymerization by the drier conditions.

Although the synthesis of nitrogenous compounds should be affected in a way similar to that of the carbohydrates, the results on the two fractions of the nitrogenous compounds as presented in table 5 do not indicate that it is. There appears to be no indication of a significantly quicker rate of protein formation in the diseased than in the normal peas. Since the nitrogen supply to diseased peas is inadequate, however, one should not expect the same behavior of proteins as of the carbohydrates, an excess of which seems to be transferred into the ovules.

All the established differences in the chemical composition of peas from normal and diseased plants have a direct bearing on the quality of the green peas as harvested for canning. The relative rate at which the sugar in the peas changes into higher carbohydrates determines the changes in quality and thus the time at which the crop is in prime condition for canning. Therefore, the fact that there was a higher total carbohydrate and lower sugar content in the diseased than in the normal peas indicated essential differences in quality between the two lots.

SUMMARY

The chemical composition of peas harvested from plants affected by the root-rot complex is different from that of normal plants of the same age and history grown in the same field.

The injured root system seems to curtail the water, nitrogen, and ash supply, which results in the setting of peas with a lower water content and a lower ash and nitrogen content in the dry matter.

The disease seems to favor quicker translocation of carbohydrates into the ovules. This may explain the abnormal enlargement of the peas from diseased plants as compared with those from normal plants.

The larger proportion of higher carbohydrates in diseased peas is presumably due to the hastening of polymerization by the lower water content.

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A COMPARISON OF METHODS OF BOTANICAL ANALYSIS OF THE NATIVE PRAIRIE IN WESTERN NORTH DAKOTA [1]

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INTRODUCTION

In grassland investigations it is becoming increasingly necessary to use more refined methods of botanical analysis than those which were adequate for the early pioneering studies. One or more methods may be selected for a particular investigation, but usually data collected by 2 or 3 methods will form a firmer foundation for conclusions than data secured by only 1 method. Observational notes, in detail, are always necessary but should be supplemented by statistical data.

The purpose of this paper is to compare and evaluate the various methods of botanical analysis which have been tested in the native prairie of western North Dakota. Such a study and others that have been made, particularly at Aberystwyth,[3] and in Australia (*3*, *4*, *7*),[4] are essential in the development of grassland technic.

The field work was done during the summers of 1932 and 1933 in the vicinity of Sentinel Butte in Golden Valley County, N. Dak. The vegetation belongs to the type classified by Clements (*9*, *p. 464*) as "mixed prairie", consisting of the taller dominant grasses *Stipa comata* Trin. and Rupr. and *Agropyron smithii* Rydb., and the shorter dominant grasses and sedges *Bouteloua gracilis* Lag., *Carex filifolia* Nutt., and *C. stenophylla* Wahl. This type, with minor changes in the dominants, is widespread in the northern Great Plains and along the foothills of the Rocky Mountains.

DESCRIPTION OF PLOTS AND SAMPLE AREAS

The criteria used in selecting the chief experimental area were: Extent sufficient for distribution of plots; vegetation as typical and homogeneous as possible; topography and soils as uniform as possible; and freedom from erosion, mowing, and grazing, or other disturbing factors. After considerable reconnaissance a fairly level plateau top, comprising about 8 acres, was selected. A shallow depression, in which forbs [5] were more numerous than elsewhere, ran across one side of it. The soil was usually a heavy clay loam, dark

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[4] Reference is made by number (italic) to Literature Cited, p. 842.

[5] The term "forbs" is used to denote herbs other than grasses.

brown in color. It did not become lighter in color until a depth of about 12 inches was reached. In places the soil varied to a silt clay and in other places to a lighter textured clay loam which changed to pale brown at a depth of 3 or 4 inches. *Agropyron smithii* was more abundant, and *Carex filifolia* and *Stipa comata* were less abundant, on soil containing a high proportion of clay; the reverse was true on soil with a lower proportion of clay.

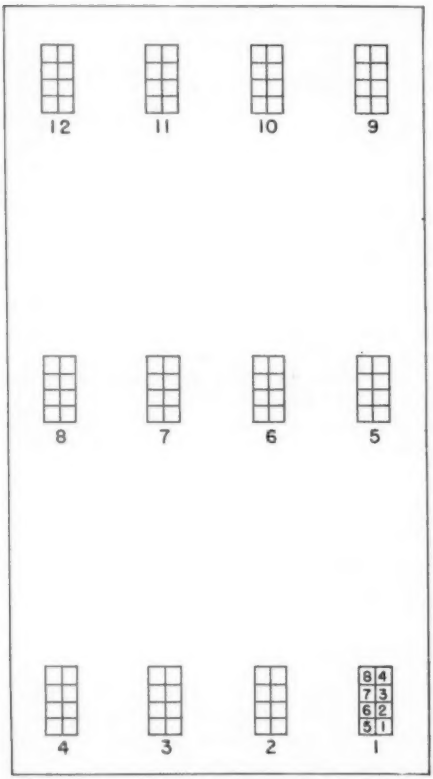

FIGURE 1.—Diagram showing distribution of plots. Each plot was 4 rods long and 2 rods wide. The distance between plots 1 and 2, 2 and 3, etc., was 150 feet; between plots 1 and 5, 2 and 6, etc., 500 feet.

Twelve plots were distributed systematically, as shown in figure 1. A wide margin was allowed on each side of the area before the sides of the plateau were reached. Each plot was subdivided into 8 square rods. Sample areas were located at random near the center of each quarter of each square rod. A wooden sampling frame 8 dm long and 5 dm wide, or 0.4 m^2, was subdivided into 4 parts, each 2.5 dm wide and 4 dm long, or 0.1 m^2. Data were collected separately for each 0.1 m^2 area, making a total of 16 sample areas per square rod, 128 per plot, and 1,536 for the 12 plots. These small sample areas and the square-rod units in the plots were combined in various ways so as to form several sizes and shapes of sampling areas and plots.

METHODS

Four criteria of the nature of grassland vegetation were used in the botanical analyses. They were area covered, number of stalks or plants, frequency, and weight (productivity) of species per unit of soil surface. The methods employed for determining extent of area covered were the "area-list" method (*3*), in which the listing square (*6*) and estimation were used, and the point-quadrat method (*7*). The "count-list" method (*3*), similar to the percentage-frequency method of Davies,[6] was employed for securing data on number of stalks or plants. The frequency method as applied to grazing studies

[6] DAVIES, W. See footnote 3.

has been described by Hanson, Love, and Morris (*5*) and corresponds to Stapledon's specific-frequency method referred to by Davies. Dry weight was secured by the "list-weight" method (*3*), which is similar to the percentage productivity method described by Davies.

AREA-LIST AND COUNT-LIST METHODS

Because of the large amount of work required in collecting and analyzing the data, observations were limited to counting the number of stalks of *Agropyron smithii*, and estimating the basal area, with the aid of the listing square, of *Bouteloua gracilis* and *Stipa comata*, and the percentage of total plant cover or density. This was done for the total number, 1,536, of 0.1 m^2 sample areas on the 12 plots.

Degree of Heterogeneity in the Experimental Area

The degree of heterogeneity of the vegetation was determined by various statistical methods. The distribution of the 128 samples of each of the 3 species and the total density of vegetation are shown for each plot by means of frequency polygons in figure 2. It is apparent that there was considerable variation within and between plots. As indicated by the similarity in the form of the polygons on the different plots, there is greater uniformity on the experimental area in "density" and in the distribution of *Bouteloua* than in the distribution of the other two grasses. *Agropyron* is next in order, but there is an especially high order of skewness in plots 3, 4, and 12 as compared with the other plots. Because of the sparseness of *Stipa* on most of the experimental area the distribution polygons are considerably skewed except plot 3, which shows more symmetry than any of the other plots. The shape of the polygons and the field observations indicate that because of its fairly high degree of uniformity in distribution and abundance on the experimental area, *Bouteloua* was influenced least by variations in soil factors; that conditions were most favorable for the growth of *Agropyron* on plots 1, 2, 5, 6, 9, 10, and 11 and least favorable on plots 3, 4, and 12; and that conditions were most favorable for growth of *Stipa* on plots 3, 4, and 12. There appears to be little doubt that soil conditions, such as high clay content, favorable for *Agropyron*, were unfavorable for *Stipa*, and that these variations were not large enough to influence greatly the distribution of *Bouteloua*. Plot 2 was located in a shallow depression, and in plot 3 the soil was of a lighter texture than in the other plots. The evaluation of various factors, such as differences in soil conditions and growth habits of species upon the heterogeneity of the vegetation, needs further investigation.

The conclusions derived from field observations and from study of the frequency polygons are supported by comparison of the means and probable errors of the means of the one hundred and twenty-eight 0.1-m^2 sample areas on each of the 12 plots. This comparison is presented graphically in figure 3. As indicated by the height of the means (A) columns, the best stands of *Agropyron* were on plots 1, 2, 5, 6, 9, 10, and 11; the poorest on plots 3, 4, 7, 8, and 12. The best stands of *Bouteloua* were on plots 1, 9, 11, and 12, and the poorest on plot 3. The best stands of *Stipa* were on plots 3, 4, and 12, and the poorest on plot 1. The uniformity in both the means and

the percentages of probable errors of the means for density is due partly to the method (estimation) employed in the field, but mostly to the fair degree of homogeneity of the cover of *Bouteloua*. Decreases in *Agropyron*, as in plots 3 and 12, were compensated for in the total cover, in part, by increases in *Stipa* or *Bouteloua*. The variation within the plots, as indicated by the probable errors, was least for

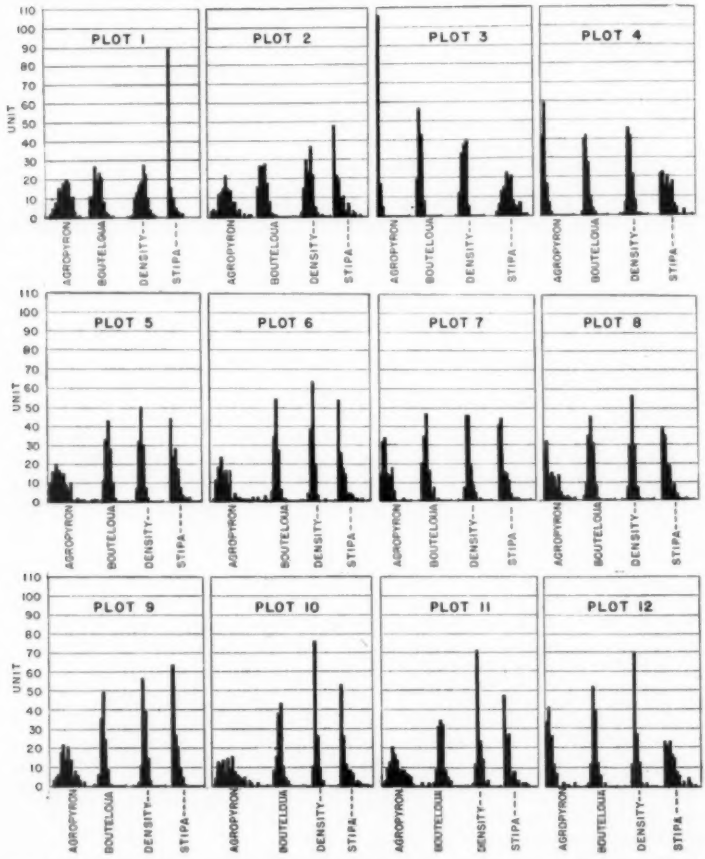

FIGURE 2.—Frequency histograms of number of stalks of *Agropyron smithii*, square centimeters of *Bouteloua gracilis* and *Stipa comata*, and percentage of density (total basal cover) on one hundred and twenty-eight 0.1 m² sample areas per plot. Class intervals are 5 for *Agropyron*, 50 for *Bouteloua*, 5 for density, and 3 for *Stipa*.

Agropyron in plots 1, 2, 9, 10, and 11, and greatest in plots 3, 8, and 12. For *Bouteloua* the variation was fairly low in all of the plots, but lowest in 6, 9, and 12. In the case of *Stipa* the variation within plots was fairly high in all plots except 3 and 4.

The dissimilarity of the plots was also studied by determining the significance of the differences between the means of different plots.

The means of all the samples of *Bouteloua*, *Agropyron*, and *Stipa* in each plot were used. A ratio was secured for every combination of 2 plots by dividing the difference between the means of the 2 plots by the probable error of the difference, according to the formula $R=\frac{m^1-m^2.}{p.\ e.\ d.}$ When the resulting ratio is 3 or less the degree of similarity tends to be high. The higher the ratio is above 3, the smaller is the similarity in the means between the two plots. For the area as a whole there was much greater similarity between the

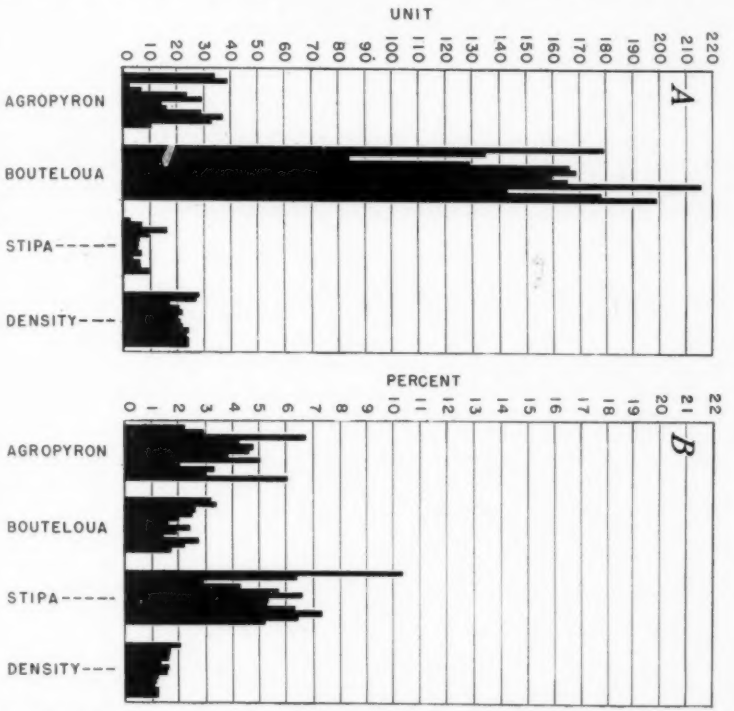

FIGURE 3.—Means (*A*) and probable errors (*B*) of the means for each of the 12 plots of the number of stalks of *Agropyron smithii*, square centimeters of *Bouteloua gracilis* and *Stipa comata*, and percentage of density (total basal cover) on 128 0.1-m² sample areas. For each grass and for density each of the 12 plots is represented by an individual column with plot no. 1 at the left in each set.

plots for *Bouteloua* and *Stipa* than for *Agropyron*. In a total of 60 plot comparisons for each grass, *Bouteloua* had 19 with a ratio of 3 or less, *Stipa* 22, and *Agropyron* 8. The similarity of the plots in the case of *Stipa* was due largely to the extreme scarcity of this grass in most of the plots (fig. 2). In only 4 sets of plots (2–10, 5–6, 6–11, 7–8) did each of the 3 grasses show a high degree of similarity. The similarity of the plots can also be studied by examination of the frequency polygons (fig. 2), but it is more difficult because of the number of columns that must be considered.

The coefficient of variation $\left(\frac{\sigma \times 100}{\text{Mean}}\right)$ was secured for each of the 12 plots for number of stalks of *Agropyron*, square centimeters of *Bouteloua* and *Stipa*, and percentage of density of one hundred and twenty-eight 0.1-m² sample areas per plot. The coefficients were lowest for density and for *Bouteloua*, intermediate for *Agropyron*, and highest for *Stipa*, which indicated increasing heterogeneity in the order given. For distribution within a plot the greatest homogeneity for *Agropyron* was found in plots 9, 1, 2, 10, and 11; for *Bouteloua* in 9, 6, 12, 5, 11, 7, 4, 3, and 10; for *Stipa* in 3; for density in 10, 9, 12, 5, 8, and 11. Considering all species, the most homogeneous plots, in decreasing order, were 9, 4, 11, 7, and 6.

In order to determine whether there was any relationship in the abundance of *Bouteloua* and *Agropyron* on the 12 plots, the coefficient of correlation was calculated. The data given in tables 3 and 4 (384 sample areas) were used in the calculations. The coefficient of correlation was -0.09 ± 0.034, showing that there was no relationship between the two; for example, if *Agropyron* decreased *Bouteloua* showed no significant tendency either to increase or decrease.

Size, Shape, and Number of Sample Areas

The following sizes and shapes of sample areas were compared: 0.1-m² (2.5 by 4 dm), 0.2-m² (2.5 by 8 dm and 5 by 4 dm), and 0.4-m² (5 by 8 dm). The percentage of probable error of the mean was secured for various numbers of sample plots (randomly selected) in each of the 12 plots. The averages of the 12 probable errors for *Agropyron*, *Bouteloua*, and *Stipa* are shown in table 1.

Table 1.—*Average probable errors of the mean in percentage of the various numbers of sample areas of different sizes and shapes for 12 plots (2 by 4 rods)*

Sample areas per plot			Average probable error		
Number per plot	Size	Shape	*Agropyron smithii*	*Bouteloua gracilis*	*Stipa comata*
	m²	*Dm*	*Percent*	*Percent*	*Percent*
8	0.1	2.5 by 4	13.6	8.4	18.8
8	.2	2.5 by 8	13.6	6.6	15.8
16	.1	2.5 by 4	11.3	6.5	15.4
32	.1	2.5 by 4	7.7	4.3	10.5
32	.2	2.5 by 8	7.0	3.8	----------
32	.2	5.0 by 4	7.4	4.0	----------
32	.4	5.0 by 8	5.6	3.5	8.8
128	.1	2.5 by 4	4.0	2.3	6.0

If the percentage of probable error of the mean is used as a criterion, the data in table 1 indicate that the 0.1-m² size is preferable to the other sizes and shapes for the following reasons: When the same number of sample areas is used the probable error of the 0.2-m² size is not enough lower than the 0.1-m² size to justify the additional time required for field work. The sixteen 0.1-m² sample areas give lower probable errors than eight 0.2-m² areas, and one hundred and twenty-eight 0.1 sample areas give better results than thirty-two 0.4 areas. Therefore for the same total area of samples it is better to use

higher numbers of the 0.1-m² sample area than lower numbers of the larger sizes. The 2.5- by 8-dm sample area usually gave slightly lower probable errors than the 2.5 by 4 shape, when the same numbers were used for each.

In determining the lowest number of sample areas that should be used, comparisons were limited to the 0.1-m² size. Probable errors in percentage of the mean were calculated for different numbers of sample

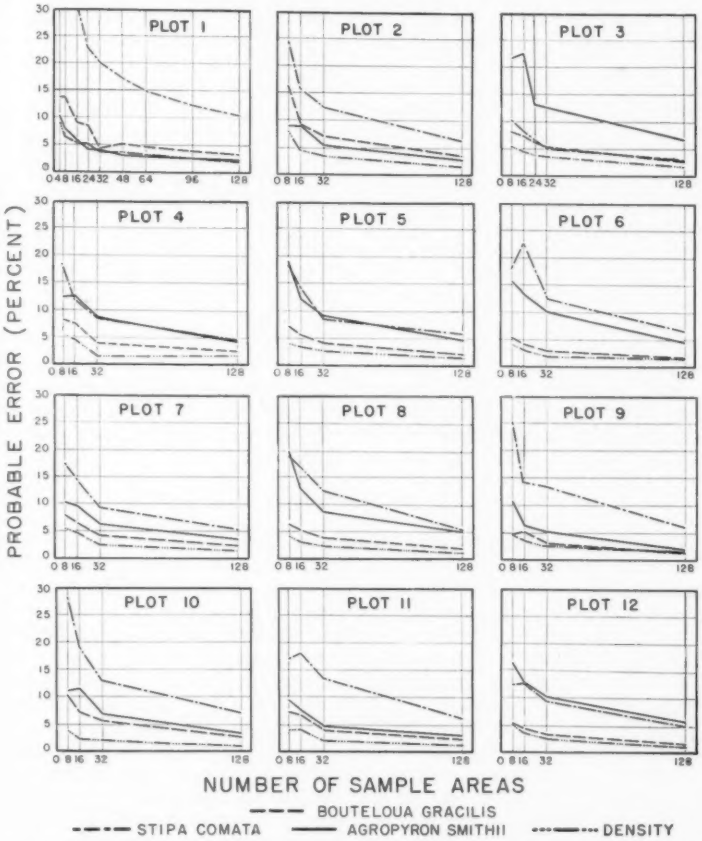

FIGURE 4. Percentages of probable error of the mean for different numbers of sample areas per plot for *Agropyron smithii, Bouteloua gracilis, Stipa comata,* and density (basal cover).

areas selected at random in each plot. The graphs representing these probable errors are given in figure 4. Each percentage of probable error for 4 sample areas per plot is the average of the probable errors of 6 random selections of 4 each. When 8 sample areas were used 4 random selections were averaged, for 16 areas 3 were averaged, for 24 areas, 2, and for higher numbers of sample areas only 1 random selection was used.

A study of the graphs in figure 4 reveals that for *Bouteloua* 8 sample areas per plot are usually sufficient, since in only 3 plots does the probable error of 8 samples exceed 10 percent. When 16 sample areas are used per plot all of the probable errors are below 10 percent. For *Agropyron* and *Stipa* 32 sample areas per plot appear to be more satisfactory as a minimum number than 16 or 8. Even when 32 are used there are 3 plots for *Agropyron* and 7 for *Stipa* in which the probable errors exceed 10 percent. Most of the curves in figure 4 show steep declines from 4 or 8 samples to 32 samples. The curves are much more gradual after the 32 point has been reached. The investigator would be well repaid in the decrease of the probable error if he increased his number of sample areas up to 32, but when this number is passed he would usually have to use an extremely large number of sample areas in order to secure only a small decrease in probable error. The large amount of time and labor required to secure the additional small decrease would not usually be justified by the slight increase in accuracy. If only *Bouteloua* were being studied, 8 to 16 sample areas per plot would be sufficient. Since in most investigations it would be necessary to include both *Agropyron* and *Stipa* in addition to *Bouteloua* it appears that the minimum number of sample areas to use per plot (2 by 4 rods) would be 32.

The graphs in figure 4 show that the lowest probable errors, and hence the most homogeneous distribution within the plot, occurred for *Bouteloua* in plots 6, 9, and 12; for *Agropyron* in plots 1, 2, 9, and 11; and for *Stipa* in plots 3 and 12. The highest probable errors and most heterogeneous distribution within the plot were found for *Bouteloua* in plots 1, 2, and 10; for *Agropyron* in plots 3, 6, 8, and 12; and for *Stipa* in plots 1, 2, 6, 8, 9, 10, and 11.

Size and Shape and Number of Plots

Six sizes and shapes of plots were used; namely, 1 by 1 rod, 1 by 2 rods, 1 by 4 rods, 2 by 2 rods, 2 by 3 rods, and 2 by 4 rods. The means and their probable errors for different numbers of sample areas for plots 9 and 3 are presented in table 2. From the 12 plots these 2 were selected because they represented important differences. Plot 9 had high means for *Agropyron* and *Bouteloua*, but low for *Stipa*. Plot 3 had low means for *Agropyron*, fairly low for *Bouteloua*, and high for *Stipa* (figs. 2 and 3).

Table 2 shows that there is no consistent decrease in the probable error as the size of plot increases, but that there is a considerable decrease in probable error as the number of sample areas per plot is increased from 8 to 32. In many cases the probable error is lower for the 1- by 1-rod plot than for any of the other sizes. This is to be expected because usually the smaller the area the greater is the homogeneity of the vegetation. It does not follow, however, that this size of plot is the best to use, for a large number of such plots would be required in order to obtain reliable information regarding the vegetation on the experimental area, and the use of large numbers of plots would not be practical because of the expense required to fence enclosures and to perform field operations such as harrowing and drilling. Since there is not much difference between the probable errors of the various sizes of plots it appears advisable, therefore, to recommend the use of a plot similar in shape to the largest size that was tested, i.e., 2 by 4 rods. This size would give a truer representa-

tion of the heterogeneity of the vegetation and would be well adapted to various kinds of field treatment. It is possible that a larger size might be even better, but the necessary data are not available.

TABLE 2.—*Means and probable errors of the mean for different sizes and shapes of plots and for different numbers of sample areas, 0.1 m², per plot*

Plot and species	Sample areas	1 by 1 rod		1 by 2 rods		1 by 4 rods		2 by 2 rods		2 by 3 rods		2 by 4 rods	
		Mean	Probable error	Mean	Probable error	Mean	Probable error	Mean	Probable error	Mean	Probable error	Mean	Probable error
Plot No. 9:													
Agropyron Smithii, stalks	*Number*		*Pct.*		*Pct.*		*Pct.*		*Pct.*		*Pct.*		*Pct.*
	8	28.4	7.0	22.2	10.4	29.9	7.7	23.8	12.2	32.2	10.5	27.9	10.6
	16	29.9	4.6	23.9	5.7	29.5	6.1	25.7	7.0	28.1	8.5	29.4	6.4
	32	------	------	22.5	5.1	28.6	5.1	24.9	5.5	30.4	4.8	28.1	5.1
Bouteloua gracilis, square centimeters	8	229.5	4.1	216.7	5.7	213.3	4.9	217.5	6.4	246.7	6.5	222.5	4.8
	16	228.7	2.8	229.4	4.1	211.9	3.7	204.4	3.1	220.0	3.6	212.8	5.2
	32	------	------	220.6	2.8	216.9	2.2	204.7	3.0	223.7	2.5	215.9	3.0
Stipa comata, square centimeters	8	3.7	27.0	4.5	17.3	3.9	16.8	4.0	27.4	2.0	25.0	3.7	25.3
	16	4.0	16.0	4.4	15.2	3.0	15.3	4.1	18.8	4.3	13.0	4.4	14.3
	32	------	------	4.4	11.7	4.6	10.9	4.6	11.7	3.0	11.2	3.3	13.5
Plot No. 3:													
A. smithii, stalks	8	1.1	40.0	3.0	16.6	2.9	20.4	3.8	17.7	2.4	16.1	3.2	21.6
	16	1.2	33.0	2.4	14.9	2.5	18.5	2.9	12.3	2.6	21.0	2.0	22.5
	32	------	------	2.6	9.3	2.9	12.0	2.6	11.9	2.5	12.7	2.6	12.6
B. gracilis, square centimeters	8	84.1	5.4	58.6	12.1	82.1	12.4	84.3	10.6	83.7	14.4	90.9	8.0
	16	84.4	4.0	65.6	6.6	85.3	7.7	78.5	6.5	86.7	8.9	83.0	7.3
	32	------	------	62.6	6.0	82.3	5.5	71.5	6.2	77.9	5.1	83.4	5.2
S. comata, square centimeters	8	11.4	10.1	19.7	8.4	18.3	10.9	15.4	9.1	17.4	9.9	16.7	10.2
	16	12.2	6.8	22.5	6.4	17.1	8.0	16.0	6.9	13.9	9.1	17.0	8.1
	32	------	------	20.6	4.8	17.4	5.6	17.8	4.5	16.2	5.0	15.3	5.0

Comparisons of sizes and shapes of plots were also made by means of Fisher's method of variance (*1*). It was deemed sufficient to use only the observations on *Bouteloua* and *Agropyron*. In order to make the comparison as strictly applicable as possible to size and shape only, the same sample areas and plots were used throughout except as additional ones had to be added because of increase in size of the plot. In each case four 0.1-m² sample areas per square rod were used. Since there were available 4 sample areas in each quarter of each square rod, 1 of each set of 4 was selected at random. The same sample areas served for both *Bouteloua* and *Agropyron*. When the size of the plot was 1 by 2 rods, square rods 1 and 2 in each of the large plots were used. When the size of plot was 1 by 4 rods (square rods 1, 2, 3, and 4 in each large plot) it was necessary to select 16 sample areas in order to maintain the same ratio of 4 sample areas to each square rod. The same number, 16, of sample areas was selected when the size and shape was 2 by 2 rods (square rods 1, 2, 5, 6). Similarly, when the size was 2 by 3 rods (square rods 1, 2, 3, 5, 6, 7) 24 sample areas were used, and for the size 2 by 4 rods (all available square rods, 1 to 8) 32 sample areas were used. By this procedure the number of sample areas increased as the size of plot increased in order to maintain the same areal ratio and to eliminate the error that would arise from fewer sample areas per unit of soil surface. The data are presented in tables 3 and 4, and calculations from the data made by the procedure outlined by Snedecor (*8*), are shown in table 5.

TABLE 3.—*Number of stalks of Agropyron smithii from 384 sample areas, each 0.1 m², in plots 2 by 4 rods*

Square rod no.	Sample area no.	Number of stalks of *A. smithii* from plot no.—												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
1	1	36	11	0	0	24	38	25	22	33	30	14	0	
	2	39	33	3	0	9	25	14	2	26	44	28	4	
	3	25	39	5	5	42	39	32	21	20	96	40	2	
	4	38	35	5	5	12	46	11	0	20	43	56	3	
2	5	36	38	0	0	0	10	14	3	36	32	39	4	
	6	37	48	1	8	18	12	9	8	33	32	45	22	
	7	53	46	3	6	3	14	18	0	32	23	41	3	
	8	54	31	2	11	9	24	7	9	29	26	40	5	
3	9	57	33	2	5	8	14	17	9	19	20	27	0	
	10	33	17	3	8	8	23	28	2	30	43	54	12	
	11	51	30	5	3	25	13	3	5	29	37	47	9	
	12	30	21	0	10	40	16	11	4	5	54	25	18	
4	13	20	29	2	16	7	5	20	7	49	84	63	9	
	14	11	15	0	6	60	9	5	30	34	23	22	15	
	15	30	19	0	2	25	20	2	3	28	58	21	14	
	16	20	64	0	7	56	24	28	43	0	33	17	10	
5	17	13	61	0	5	19	28	12	11	66	27	38	2	
	18	45	38	0	4	33	15	6	18	57	50	30	1	
	19	54	55	4	1	32	28	8	3	18	95	40	12	
	20	38	26	3	4	15	32	17	16	48	50	21	44	
6	21	17	37	0	18	3	69	6	6	52	68	24	5	
	22	24	57	0	0	21	50	13	19	17	15	31	5	
	23	22	19	5	3	41	44	8	67	34	20	13	21	
	24	35	42	7	3	32	35	25	21	37	23	58	15	
7	25	40	79	3	5	20	28	6	12	22	16	44	8	
	26	43	19	1	8	31	4	21	14	21	32	31	8	
	27	32	27	0	7	26	18	18	9	38	17	63	7	
	28	47	63	10	12	35	16	19	16	30	12	18	6	
8	29	23	26	6	7	10	31	6	31	32	15	24	11	
	30	14	64	2	5	35	35	1	30	26	34	26	13	
	31	42	60	1	7	43	17	16	34	24	36	5	13	
	32	35	67	2	3	108	38	8	29	14	31	6	13	
Total		1,094	1,249	75	184	850	820	434	504	959	1,219	1,051	314	8,753

TABLE 4.—*Number of square centimeters of Bouteloua gracilis from 384 sample areas, each 0.1 m², in plots 2 by 4 rods*

Square rod no.	Sample area no.	Number of square centimeters of *B. gracilis* from plot no. —												Total
		1	2	3	4	5	6	7	8	9	10	11	12	
1	1	250	80	40	120	180	260	130	190	260	140	240	180	
	2	200	160	40	80	110	160	120	130	240	120	240	200	
	3	220	100	80	80	80	120	80	120	210	100	120	150	
	4	150	0	10	220	180	170	80	190	250	150	200	220	
2	5	150	160	80	80	270	190	80	230	190	140	100	200	
	6	100	150	50	170	200	210	200	100	210	150	180	180	
	7	200	120	80	180	110	100	300	160	300	80	190	140	
	8	170	140	70	90	120	140	120	100	200	340	160	250	
3	9	200	280	130	80	180	220	210	150	160	90	90	200	
	10	100	200	60	120	140	110	140	100	200	40	170	160	
	11	300	180	80	160	140	170	180	120	150	60	120	310	
	12	180	150	110	100	240	150	60	150	250	80	150	190	
4	13	230	200	80	80	200	120	110	110	180	20	160	230	
	14	100	200	110	80	270	140	150	180	150	30	160	230	
	15	100	180	100	110	180	190	120	180	170	30	380	240	
	16	250	260	70	60	180	140	130	220	190	180	120	190	
5	17	230	80	30	100	120	110	160	190	180	150	100	210	
	18	120	40	150	250	200	270	200	160	290	120	60	250	
	19	500	40	80	320	40	150	240	140	270	200	120	200	
	20	120	20	120	140	70	140	270	80	280	160	110	160	
6	21	190	30	80	80	170	140	170	200	170	220	180	190	
	22	250	30	100	80	170	250	180	200	230	150	120	210	
	23	200	100	90	190	150	250	210	250	240	280	80	280	
	24	180	100	100	110	230	200	200	280	240	160	120	150	
7	25	40	60	120	130	70	150	120	200	210	120	150	170	
	26	250	180	80	110	170	160	150	130	220	80	240	170	
	27	180	30	100	230	270	130	300	170	220	220	190	220	
	28	200	140	120	90	100	160	160	150	170	160	200	80	
8	29	120	200	50	100	210	210	130	120	220	110	280	160	
	30	200	120	120	100	240	190	110	100	240	160	320	140	
	31	80	150	100	40	160	150	80	200	310	110	310	170	
	32	90	250	60	90	100	140	90	150	160	180	210	180	
Total		5,850	4,130	2,690	3,970	5,250	5,390	4,980	5,240	6,960	4,330	5,570	6,210	60,570

TABLE 5.—*Analysis of variance of number of stalks of Agropyron and square centimeters of Bouteloua in different sizes or shapes of plots, 12 plots used in each case*

Sample areas, plots, and variation	Degrees of freedom	Sum of squares		Mean square		Standard deviation [1]	
		Agropyron smithii	*Bouteloua gracilis*	*Agropyron smithii*	*Bouteloua gracilis*	*Agropyron smithii*	*Bouteloua gracilis*
8 sample areas in plots 1 by 2 rods:							
Between means of square rods	7	1,350.96	18,445.84	192.99	2,635.12	7.86	38.44
Between means of plots	11	19,208.46	168,670.84	1,746.22	15,333.71		
Within plots (error)	77	9,509.54	227,579.16	123.50	2,955.57		
Total	95	30,068.96	414,695.84				
16 sample areas in plots 1 by 4 rods:							
Between means of square rods	15	1,768.50	32,436.99	117.90	2,162.46	6.61	30.73
Between means of plots	11	31,227.29	277,910.42	2,838.84	25,264.58		
Within plots (error)	165	28,926.69	523,572.91	175.31	3,173.16		
Total	191	61,922.48	833,920.32				
16 sample areas in plots 2 by 2 rods:							
Between means of square rods	15	2,539.67	34,914.59	169.31	2,327.64	6.78	30.09
Between means of plots	11	35,598.50	368,047.92	3,236.23	33,458.90		
Within plots (error)	165	30,412.50	597,835.41	184.32	3,623.24		
Total	191	68,550.67	1,000,797.92				
24 sample areas in plots 2 by 3 rods:							
Between means of square rods	23	3,276.42	103,415.29	142.45	4,496.32	5.43	23.94
Between means of plots	11	47,708.12	382,365.28	4,337.10	34,760.48		
Within plots (error)	253	44,765.13	870,318.05	176.94	3,439.99		
Total	287	95,749.67	1,356,098.62				
32 sample areas in plots 2 by 4 rods:							
Between means of square rods	31	4,666.34	113,738.16	150.52	3,668.97	5.12	21.66
Between means of plots	11	58,057.65	316,237.47	5,277.96	28,748.86		
Within plots (error)	341	71,645.76	1,280,188.28	210.10	3,754.21		
Total	383	134,369.75	1,710,163.91				

[1] Calculated by taking the square root of the mean square of the error divided by the number of square rods used in each plot, e. g., $\sqrt{\frac{2,955.57}{2}}=38.44$.

The analysis of variance as shown in table 5 reveals that the mean square between means of plots is greater in every case than the mean square between the means of the square rods or within the plots. This indicates that there were greater differences in the abundance of *Agropyron* and of *Bouteloua* from plot to plot than from 1 square rod to another or between the sample areas within a single plot.

The standard deviations were calculated to determine, if possible, the best size and shape of plot to use. In the case of *Bouteloua* the range in the standard deviation was from 38.44 for the 1 by 2 rod plot to 21.66 for the 2 by 4 rod size. The range in *Agropyron* was from 7.86 to 5.12 for the same sizes. In both *Bouteloua* and *Agropyron* the decrease in the standard deviation was large from the 1 by 2 rod size to the 1 by 4 rod size and from the latter to the 2 by 3 rod size,

but from the 2 by 3 rod size to the 2 by 4 rod size the decrease was small. This analysis, then, shows that the minimum size of plot that would be desirable to use is the 2 by 3 rod one. The reason for this is that the rate of decrease in the standard deviation as the size of plot increases is rapid and fairly regular until this size is reached; but when a larger size is used the rate of decrease is too slow to warrant the extra work required. No significant differences were found in the standard deviations between the rectangular shape of plot measuring 1 by 4 rods and the square shape, measuring 2 by 2 rods.

In order to determine the minimum number of plots (2 by 3 rods and 2 by 4 rods in size) that would be necessary to secure reliable results, calculations of variance were also made for 12 additional sets of field observations, for each size of plot, namely, 2 random selections of data for 5 plots, 2 for 7 plots, and 2 for 10 plots, for *Agropyron* and *Bouteloua* separately. The same sample areas shown in tables 3 and 4 were used, so that the mean squares would be directly comparable to those in table 5. The analysis is presented in table 6.

TABLE 6.—*Analysis of variance of number of stalks of Agropyron and square centimeters of Bouteloua for different numbers of plots (each 2 by 3 rods in size and 24 or 32 0.1-m² sample areas in each)*

24 SAMPLE AREAS

Plots and variations	Degrees of freedom	Sum of squares		Mean square		*F* value [1]	
		Agropyron smithii	*Bouteloua gracilis*	*Agropyron smithii*	*Bouteloua gracilis*	*Agropyron smithii.*	*Bouteloua gracilis*
5 plots, nos. 1, 8, 9, 10, 12:							
Between means of plots	4	23,741	86,895	5,935	21,724		
Within plots	115	20,220	432,604	176	3,762	32.9	5.8
Total	119	43,961	519,499				
5 plots, nos. 2, 3, 7, 9, 11:							
Between means of plots	4	22,479	284,542	5,620	71,135		
Within plots	115	16,551	339,925	144	2,956	39.0	24.1
Total	119	39,030	624,467				
7 plots, nos. 3, 5, 6, 7, 10, 11, 12:							
Between means of plots	6	25,581	173,475	4,264	28,912		
Within plots	160	29,008	498,196	181	3,114	23.5	9.3
Total	167	54,589	671,671				
7 plots, nos. 1, 2, 4, 5, 7, 9, 11:							
Between means of plots	6	23,446	215,566	3,908	35,928		
Within plots	160	23,857	663,584	149	4,147	26.2	8.7
Total	167	47,303	879,150				
10 plots, nos. 1, 2, 3, 4, 5, 7, 8, 10, 11, 12:							
Between means of plots	9	45,546	263,876	5,060	29,320		
Within plots	230	37,854	870,421	165	3,784	30.7	7.7
Total	239	83,400	1,134,297				
10 plots, nos. 1, 2, 4, 5, 6, 7, 8, 10, 11, 12:							
Between means of plots	9	58,807	148,969	6,534	16,552		
Within plots	230	43,065	910,950	187	3,961	34.9	4.2
Total	239	101,872	1,059,919				
12 plots, all numbers:							
Between means of plots	11	48,398	382,816	4,400	34,801		
Within plots	276	119,114	976,134	432	3,537	10.2	9.8
Total	287	167,512	1,358,950				

[1] The *z* values given by Fisher (*7*) are equal to one-half the natural logarithms of the values of Snedecor (*8*).

TABLE 6.—*Analysis of variance of number of stalks of Agropyron and square centimeters of Bouteloua for different numbers of plots (each 2 by 3 rods in size and 24 or 32 0.1-m² sample areas in each)*—Continued

32 SAMPLE AREAS

Plots and variations	Degrees of freedom	Sum of squares		Mean square		*F* value	
		Agropyron smithii	*Bouteloua gracilis*	*Agropyron smithii*	*Bouteloua gracilis*	*Agropyron smithii*	*Bouteloua gracilis*
5 plots, nos. 1, 8, 9, 10, 12:							
Between means of plots	4	22,043	123,659	5,510.75	30,914.7		
Within plots	155	35,195	566,565	227.1	3,655.3	24.3	8.5
Total	159	57,238	690,224				
5 plots, nos. 2, 3, 7, 9, 11:							
Between means of plots	4	29,334	401,750	7,333.5	100,437.5		
Within plots	155	27,007	475,670	174.2	3,068.8	42.1	32.7
Total	159	56,341	877,419				
7 plots, nos. 3, 5, 6, 7, 10, 11, 12:							
Between means of plots	6	32,411	241,905	5,401.8	40,317.5		
Within plots	217	47,974	723,294	221.1	3,333.2	24.4	12.1
Total	223	80,385	965,198				
7 plots, nos. 1, 2, 4, 5, 7, 9, 11:							
Between means of plots	6	27,664	198,499	4,610.7	33,083.2		
Within plots	217	47,561	985,622	219.2	4,542.0	21.0	7.3
Total	223	75,225	1,184,121				
10 plots, nos. 1, 2, 3, 4, 5, 7, 8, 10, 11, 12:							
Between means of plots	9	55,834	209,936	6,203.8	23,326.2		
Within plots	310	63,817	1,339,512	205.9	4,321.0	30.1	5.4
Total	319	119,650	1,549,448				
10 plots, nos. 1, 2, 4, 5, 6, 7, 8, 10, 11, 12:							
Between means of plots	9	42,463	155,192	4,718.1	17,243.6		
Within plots	310	70,103	1,273,562	226.1	4,108.2	20.9	4.2
Total	319	112,566	1,428,755				
12 plots, all numbers:							
Between means of plots	11	60,688	233,758	5,517.1	21,250.7		
Within plots	372	112,312	1,593,930	301.9	4,284.8	17.8	5.0
Total	383	173,000	1,827,689				

A comparison of the *F* values shows that if only 5 plots are employed for 1 kind of treatment and 5 kept as checks the effects of the treatment may be obscured or magnified because of the high degree of natural variation shown to exist between 2 sets of 5 plots not influenced by difference in treatment. When 7 plots are used, there is little difference between each set of 7 plots under natural conditions so that any differences in the abundance of *Agropyron* or *Bouteloua* due to differences in treatment would be more readily revealed and could be more definitely attributed to the treatment rather than to the natural variation of distribution of the species. Seven plots appear to be ample to use for either size of plot instead of 10 or 12, because of the small degree of variation in the 2 selections of 7 plots, and because the *F* values do not decrease greatly when 10 or 12 plots are used instead of 7. Since the *F* values do not decrease much when more than 7 plots are used, it appears that 7 plots selected at random give a fair representation of the relationship existing between the variation between and within plots of these 2 grasses.

Comparisons were also made of probable errors of the averages of the means of thirty-two 0.1-m² sample areas per plot for different numbers of replications of plots. According to the data given in table 7, 7 plots of the size and shape used, 2 by 4 rods, appear to be the minimum number that it is desirable to use. One reason for this is that the probable errors in the replications of 3 vary from 19.6 to 31.3 percent and in the replications of 5 they vary from 13.6 to 20.1 percent, but when 7 replications are used the variation is much less, from 12.8 to 14.3 percent. A second reason is that the probable errors for the set of 7 replications are usually lower than those for the 5 replications and those for the 5 replications are usually lower than those for the 3.

TABLE 7.—*Average means and probable errors of the average mean of stalks of Agropyron smithii and square centimeters of Bouteloua gracilis of 1 or more sets of replications of 32 sample areas (0.1 m²) per plot (2 by 4 rods)*

Plots chosen at random (number)	*A. smithii*		*B. gracilis*		Plots chosen at random (number)	*A. smithii*		*B. gracilis*	
	Average mean of stalks	Probable error of average mean	Average mean	Probable error of average mean		Average mean of stalks	Probable error of average mean	Average mean	Probable error of average mean
	Number	*Percent*	*Square centimeters*	*Percent*		*Number*	*Percent*	*Square centimeters*	*Percent*
3	19.3	20.1	138.7	4.0	5	22.4	20.1	145.3	2.3
3	21.6	23.7	168.9	2.2	5	25.8	15.0	156.1	13.0
3	10.5	31.3	160.4	4.1	7	20.4	14.3	166.9	4.1
3	11.6	27.1	170.0	7.1	7	20.9	13.9	168.2	4.7
3	19.3	20.1	160.4	13.7	7	23.3	12.8	145.4	5.2
3	8.4	19.6	139.9	12.5	10	22.4	9.6	155.4	2.1
5	19.8	19.4	145.7	8.9	12	22.3	10.5	159.3	4.2
5	26.0	13.6	155.4	7.1					

LIST-WEIGHT METHOD

One 0.1-m² sample area in each of the 8 square rods in each of the 12 plots was clipped at a height of 0.5 inch. As the vegetation was cut it was separated into five groups—*Agropyron smithii*, *Bouteloua gracilis* and *Carex* spp., *Stipa comata*, miscellaneous grasses, and forbs. The oven-dry weights (temperature of 100° C. was used) are given in table 8. Frequency histograms of these data are presented in figure 5. The histograms for the combined weights of *Bouteloua* and *Carex* spp. are more symmetrical than any of the others. The histograms demonstrate clearly the scarcity of forbs and *Stipa* on this area.

TABLE 8.—*Oven-dry weights of 96 0.1-m² sample areas, 1 in each of the 8 square rods in each plot, grouped into 5 classes, and total*

Plot No.	Area	*Agropyron smithii*	*Bouteloua gracilis* and *Carex* spp.	*Stipa comata*	Miscellaneous grasses	Forbs	Total
	Square rods	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	1	3.5	4.2			0.01	7.7
	2	5.6	5.6			.02	11.2
	3	3.3	6.9			.02	10.2
	4	3.1	9.0	0.3	0.5		12.9
	5	3.6	6.7			.1	10.4
	6	3.3	6.5	.7			10.5
	7	2.8	2.2	1.4			6.4
	8	2.3	3.8	.5			6.6
2	1	2.6	5.5	4.2		6.8	19.1
	2	4.0	6.4			6.7	17.1
	3	6.3	6.8	1.9	.1	2.0	17.1
	4	2.8	6.4	1.0		1.1	11.3
	5	2.5	6.0	.9	.8	6.9	17.1
	6	2.9	6.5	1.7		4.9	16.0
	7	8.7	7.8	1.6		4.3	22.4
	8	7.6	7.9	2.0		1.0	18.5
3	1	.1	3.6	2.9			6.6
	2	.3	3.3	2.3		.4	6.3
	3	.3	3.6	1.1		.02	5.0
	4		5.6	1.6	.05	.6	7.8
	5	.1	4.3	1.5	.1		6.0
	6	.4	5.2	1.9		4.2	11.7
	7		3.7	1.1		.1	4.9
	8		4.3	1.5		.3	6.1
4	1	.9	8.7	.6		.3	10.5
	2	1.9	4.8	.2		.1	7.0
	3	.3	4.6	1.8		.7	7.4
	4	.8	8.2	6.8		.7	16.5
	5	.7	7.0	.7		.6	9.0
	6	1.2	7.2			.2	8.6
	7	.6	4.0	2.2		1.0	7.8
	8	2.3	9.3	1.2	.1	.1	13.0
5	1	.4	7.7	.8		.1	9.0
	2	2.6	6.1			.2	8.9
	3	4.2	6.5	1.7		.8	13.2
	4	9.3	5.5			.3	15.1
	5	1.5	6.6	.5		1.2	9.8
	6	5.9	2.7	.1		.6	9.3
	7	5.0	4.4	1.7		.3	11.4
	8	14.3	5.7	1.2		.1	21.3
6	1	12.6	7.2	.3		.5	20.6
	2	3.2	5.8	1.5	.2	.2	10.9
	3	2.6	5.9	2.6		.1	11.2
	4	2.6	6.4	1.5		.1	10.6
	5	5.1	4.7	2.5		.7	13.0
	6	5.1	8.7			.1	13.9
	7	3.1	6.1	1.0		.2	10.4
	8	6.0	6.9	2.3		.2	15.4
7	1	1.1	5.9	.6		.2	7.8
	2	1.9	6.0	.9		.7	9.5
	3	3.1	6.3	1.5		.4	11.3
	4	7.1	5.4	1.2		.2	13.9
	5	4.9	7.6	.5		.9	13.9
	6	5.9	9.3			.1	15.3
	7	2.5	5.7	.4		.4	9.0
	8	3.6	8.1	.5		.2	12.4
8	1		6.6	2.2	.2	.7	9.7
	2	.4	5.1	1.8	.5	.3	8.1
	3	1.5	4.9	1.4	1.1	.1	9.0
	4	4.0	6.7	.5		.1	11.3
	5	3.1	5.4	.6	.5	.3	9.9
	6	2.7	6.2	1.0		.2	10.1
	7	4.2	4.6	.6	.2	.2	9.8
	8	4.5	4.7	1.6		2.5	13.3
9	1	2.5	3.7	1.4		1.3	8.9
	2	3.7	5.1			.5	9.3
	3	3.4	6.2			.3	9.9
	4	1.4	8.3	.8		.9	11.4
	5	3.4	4.6			.7	8.7
	6	4.6	5.6			1.7	11.9
	7	4.5	5.3	1.1		.7	11.6
	8	2.2	4.6	.8		1.8	9.4

TABLE 8.—*Oven-dry weights of 96 0.1-m² sample areas, 1 in each of the 8 square rods in each plot, grouped into 5 classes, and total*—Continued

Plot No.	Area	*Agropyron smithii*	*Bouteloua gracilis* and *Carex* spp.	*Stipa comata*	Miscellaneous grasses	Forbs	Total
	Square rods	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
	1	6.3	6.0	5.6	3.4	3.5	24.8
	2	4.2	8.1	.....	.4	3.1	15.8
	3	10.1	4.4	.6	.2	3.0	18.3
	4	2.5	6.1	.7	1.7	2.3	13.3
10	5	3.2	6.3	.1	1.5	3.6	14.7
	6	1.3	4.6	3.3	5.9	3.4	18.5
	7	2.0	7.4	.....	2.1	1.0	12.5
	8	4.7	7.0	.4	.5	.6	13.2
	1	6.0	4.4	1.3	1.8	1.4	14.9
	2	6.7	5.7	.....	.1	2.8	15.3
	3	3.7	6.9	.3	.2	2.7	13.8
	4	1.3	8.0	.....	.....	.9	10.2
11	5	2.1	4.5	2.3	1.7	3.4	14.0
	6	13.4	5.5	.....	.....	7.6	26.5
	7	2.8	5.1	2.1	.8	.9	11.7
	8	.3	8.9	1.5	.....	1.0	11.7
	1	1.2	5.7	2.7	.2	.3	10.1
	2	1.0	6.5	3.7	.3	.2	11.7
	3	2.3	6.2	.4	1.0	.4	10.3
	4	1.7	5.3	1.8	.7	.4	9.9
12	5	7.3	5.1	.....	.....	.6	13.0
	6	1.4	6.1	1.1	.3	.5	9.4
	7	1.2	4.3	1.3	.6	2.0	9.4
	8	1.4	5.7	1.0	.6	.1	8.8

TABLE 9.—*Analysis of variance of yields in grams according to species or groups of species and totals of ninety-six 0.1-m² sample areas*

Group and variation	Degrees of freedom	Sum of squares	Mean square	*F* value
Agropyron Smithii:				
Between means of plots	11	187.3	17.03	2.9
Within plots	84	487.6	5.8	
Total	95	675.0	7.1	
Bouteloua gracilis and *Carex* spp.:				
Between means of plots	11	48.1	4.4	2.3
Within plots	84	162.3	1.9	
Total	95	210.4	2.2	
Stipa comata:				
Between means of plots	11	20.7	1.9	1.5
Within plots	84	111.7	1.3	
Total	95	132.5	1.4	
Miscellaneous grasses:				
Between means of plots	11	27.7	2.5	6.6
Within plots	84	32.3	.38	
Total	95	60.0	.6	
Forbs:				
Between means of plots	11	143.9	13.08	9.3
Within plots	84	116.8	1.4	
Total	95	260.7	2.7	
Total:				
Between means of plots	11	831.6	75.6	6.9
Within plots	84	915.9	10.9	
Total	95	1,747.5	18.4	

Means and probable errors of the means were calculated for different numbers of sample areas. For *Agropyron* the probable errors varied from 5.8 percent for the total of 96 samples, to 8 for 72 samples, 8.7 for 48 samples, 9.3 for 36 samples, 9.6 for 24 samples, 15.8 for 12 samples, and 19.8 percent for 6 samples. For *Bouteloua* and *Carex* the probable errors increased from 1.7 percent for 96 samples to 2 for

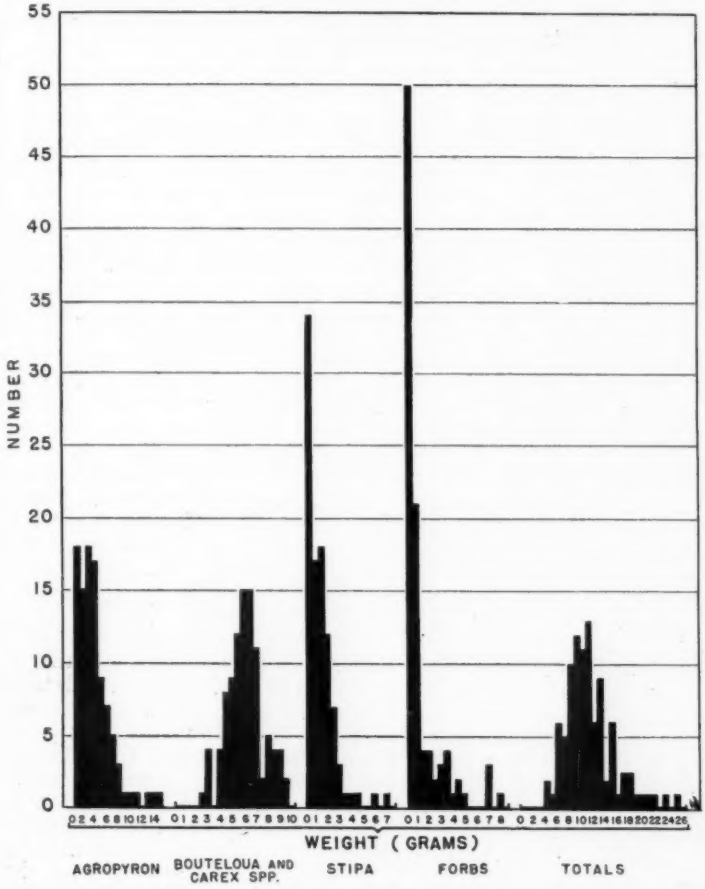

FIGURE 5.—Frequency histograms of weights according to classes and total for ninety-six 0.1-m² clipped sample areas, 1 on each of the 8 square rods in each of the 12 plots.

72, 2.5 for 36, 3.2 for 24, 4.5 for 12, and 5.1 percent for 6 samples. For total weight per sample area the range was from 2.2 percent to 7.7. For 96 samples the probable error was 9.9 percent for forbs and 3.9 for *Stipa*. These figures indicate that the lowest satisfactory number of samples for the experimental area would be 24.

An analysis of the data according to the variance method is presented in table 9. In the case of each grass or group the mean square

is larger for "between means of plots" than for "within plots." As is to be expected for native grassland, this indicates that there is more variation in the vegetation from plot to plot than within a single plot. The mean squares in the table demonstrate the important influence that the irregular distribution of the forbs and miscellaneous grasses has in causing the high degree of variation in total weights between plots. The *F* values for *Stipa*, *Bouteloua* and *Carex* spp., and *Agropyron* were low, ranging from 1.5 to 2.9, but for miscellaneous grasses the *F* value was 6.6 and for forbs it was 9.3.

Plot System Versus Scattered Sample Areas

One of the chief difficulties in quantitative grassland investigation is the high degree of heterogeneity that usually occurs, especially in natural grassland. It is important that the variation which exists on the experimental area with respect to kinds of species that are present, density, and yield be known as fully as possible before an experiment is started. With this knowledge, the experiment can be so planned that it will yield the maximum degree of reliability that the nature of the vegetation and the methods of investigation make possible. The plot system enables the investigator to determine the degree of variation that exists within small portions of the grassland and also the degree of variation that occurs between the portions or plots. By the system of scattered sample areas not located in plots, the variations may remain obscure and the grassland may be considered more homogeneous than it actually is.

It would probably be more difficult to make reliable tests of the significance of data secured from scattered sample areas than from sample areas in plots because of the great variation that frequently occurs within the area of only a few acres of vegetation. There is usually much less variation within a plot than between different plots (table 5.). A few scattered permanent quadrats are usually essential when it is necessary to study changes from year to year, but in many kinds of experiments the plot system is preferable because the data are not only better suited to statistical analysis, but include more of the variations existing in the vegetation. It appears probable that the plot system is better adapted to include in the resulting data the effects upon the vegetation of environmental influences and plant processes, as competition, in such a way that analysis of the data will lead to the discovery and evaluation of the relative importance of controlling factors. For example, the data in this paper reveal that plot 3 is strikingly different from the other plots because of the greater abundance of *Stipa* and the decreased abundance of *Agropyron* and *Bouteloua* in it. These differences might not show in a few scattered sample areas (not in plots), and if they did show, their importance might be overlooked. But by the plot system they stand out so prominently that further field study of the microrelief and of the soil is necessitated. Since exceptional conditions prevail on plot 3 as compared with the other plots, it is to be expected that any kind of treatment would have a different effect from that on the rest of the plots. Since it is desirable to have as uniform conditions as possible for experimental work, it would be desirable to omit this plot from a replication of plots to be given a certain treatment as well as from the check series. None of this procedure for the discovery,

demonstration, and special treatment of divergent portions of the experimental area is possible by the use of scattered sample areas or quadrats.

Because of the greater homogeneity within plots than between plots, as shown by the mean squares in the variance analyses (tables 5 and 9), the conclusion appears valid that the plot system is preferable to the scattered sample area method for experimental work. It is to be expected that the results obtained from plots will show less error due to heterogeneity of the vegetation than results obtained from scattered random samples because the plots on which the vegetation is widely divergent need not be included in the replications. It is also to be expected that variations in results can be more definitely assigned to differences in treatment instead of to the heterogeneity of the vegetation.

POINT METHOD

The point method for analyzing vegetation has been described in detail by Levy and Madden (*7*). This is a rapid method of securing data regarding the area of soil occupied by each species. A frame holding 10 pins, similar to the one illustrated by Levy and Madden, was used. This frame was set at 20 locations in each plot (2 by 4 rods), 10 of these locations at approximately equal distances in one of the rows of square rods in the plot and the other 10 in the other row of square rods. The total number of points per plot was 200, and since 12 plots were used the total number of points was 2,400.

The three methods described by Levy and Madden (*7*) were used to analyze the data. The first method gives the percentage of ground covered by each species or group of species. "This datum is secured by recording in each downward projection all species hit and in expressing these in terms of percentage for every 100 points examined" (*7, p. 269*). Since the foliage of different species may occupy various levels and thus cause overlapping, the sum of the percentages usually exceeds 100. The denser the vegetation and the greater the number of layers the higher will this figure be. The second method indicates the percentage of cover that each species contributes to the total area (total cover plus bare ground). It is calculated according to the formula

$$\frac{\text{Percentage of ground covered by each species}}{\text{(Total percentage of ground covered less percentage of bare ground)}} \times \text{(100 less percentage of bare ground)}$$

The third method gives the percentage that each species contributes to the pasturage. It is obtained by dividing the percentage of ground covered by each species by the total percentage of ground covered by all species (omitting bare ground).

TABLE 10.—*Analyses by the point method, showing percentages of totals contributed by each species or group of species*

Plot no. and method of analysis	*Agropyron smithii*	*Bouteloua gracilis*	*Carex filifolia*	*Carex stenophylla*	*Stipa comata*	Miscellaneous grasses and sedges	Forbs	Bare ground	Total
Plot 1:	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Ground covered	47.5	69.0	59.5	0.5	6.5	2.0	0	1.5	186.5
Total area	25.3	36.7	31.7	.3	3.5	1.0	0	1.5	100.0
Pasturage	25.6	37.3	32.1	.3	3.5	1.2	0	-------	100.0
Plot 2:									
Ground covered	34.5	81.0	.5	43.5	28.5	6.5	27.0	0	221.5
Total area	15.6	36.6	.2	19.6	12.9	2.9	12.2	0	100.0
Pasturage	15.6	36.6	.2	19.6	12.9	2.9	12.2	-------	100.0
Plot 3:									
Ground covered	3.5	67.5	40.0	7.0	29.5	0	0	8.0	155.5
Total area	2.3	40.3	25.8	4.5	19.1	0	0	8.0	100.0
Pasturage	2.5	43.8	28.1	4.9	20.7	0	0	-------	100.0
Plot 4:									
Ground covered	12.5	80.0	35.0	11.0	23.0	0	9.5	4.0	175.0
Total area	7.0	44.9	19.7	6.2	12.9	0	5.3	4.0	100.0
Pasturage	7.3	46.8	20.5	6.4	13.5	0	5.5	-------	100.0
Plot 5:									
Ground covered	24.0	82.0	9.0	23.0	14.0	1.5	9.0	3.0	165.5
Total area	14.3	48.9	5.4	13.7	8.4	.9	5.4	3.0	100.0
Pasturage	14.7	50.5	5.6	14.2	8.6	.9	5.5	-------	100.0
Plot 6:									
Ground covered	23.5	87.5	3.5	20.5	21.0	0	5.5	2.5	164.0
Total area	14.2	52.8	2.1	12.4	12.7	-------	3.3	2.5	100.0
Pasturage	14.6	54.2	2.1	12.7	13.0	0	3.4	-------	100.0
Plot 7:									
Ground covered	14.5	89.0	21.0	7.0	28.5	0	5.0	1.5	166.5
Total area	8.7	53.0	12.6	4.2	17.0	0	3.0	1.5	100.0
Pasturage	8.8	54.0	12.7	4.2	17.3	0	3.1	-------	100.0
Plot 8:									
Ground covered	19.0	76.0	27.0	5.5	19.0	.5	1.5	5.5	154.0
Total area	12.1	48.2	17.2	3.5	12.1	.3	.9	5.5	100.0
Pasturage	12.8	51.2	18.1	3.7	12.8	.3	1.1	-------	100.0
Plot 9:									
Ground covered	49.5	84.5	0	7.7	13.0	0	15.0	1.5	171.2
Total area	28.8	49.0	0	4.4	7.6	0	8.7	1.5	100.0
Pasturage	29.3	50.0	0	4.1	7.7	0	8.9	-------	100.0
Plot 10:									
Ground covered	34.5	81.0	7.5	24.0	19.5	24.0	23.5	.5	214.5
Total area	16.0	37.7	3.5	11.2	9.1	11.2	10.9	.5	100.0
Pasturage	16.1	37.9	3.5	11.3	9.0	11.2	11.0	-------	100.0
Plot 11:									
Ground covered	32.5	74.5	2.5	26.5	23.5	16.0	20.0	2.5	198.0
Total area	16.2	37.1	1.3	13.2	11.7	8.0	10.0	2.5	100.0
Pasturage	16.6	38.1	1.3	13.6	12.0	8.2	10.2	-------	100.0
Plot 12:									
Ground covered	29.5	89.0	9.0	20.0	24.5	5.5	6.5	2.5	186.5
Total area	15.6	47.2	4.8	10.6	13.0	2.9	3.4	2.5	100.0
Pasturage	16.0	48.4	4.9	10.9	13.3	3.0	3.5	-------	100.0
Average, ground covered	27.1	79.6	17.9	16.3	20.9	4.7	10.2	2.7	179.4
Average, total area contributed by each	14.9	43.8	9.9	9.0	11.5	2.6	5.6	2.7	100.0
Average, pasturage	15.0	45.7	10.8	8.8	12.0	2.3	5.4	-------	100.0

The analysis of the data by these three methods is presented in table 10 and in figure 6. Examination of the table and the figure shows considerable uniformity in the cover of *Bouteloua* in the different plots. The average of the 12 plots was 43.8 percent and the range was from 36.6 to 53. For *Agropyron* the average was 14.9 percent and the range 2.3 to 28.8. For *Stipa* the average was 11.5 percent and the range 3.5 to 19.1 *Carex filifolia* showed a slightly higher average percentage than *C. stenophylla*, 9.9 as compared to 9. Miscellaneous grasses and forbs were even lower, and "bare ground" (no hits of vegetation) gave only 2.7 percent.

Figure 6 illustrates the variation in each species or group of species that was found from plot to plot by this method. It is readily seen

that *Bouteloua* was most uniform. *Agropyron* shows much variation, plot 3 having the smallest number of hits and plots 1 and 9, the largest. By this method *Stipa* was most abundant in plots 3 and 7 and least abundant in plots 1, 5, and 10. *Carex* spp. were most abundant in plots 1, 3, and 4 and least so in plot 9. The percentage of bare ground was greatest in plots 3 and 8; least in 2 and 10. By comparison with the average, the most typical plots were nos. 12, 11, 8, 6, 5, and 2; the least typical 3, 4, 7, and 9.

According to this method almost half of the pasturage, 45.7 percent, was contributed by *Bouteloua*. When the 2 species of *Carex* are added to *Bouteloua*, the total of the 3 species becomes 65.3 percent.

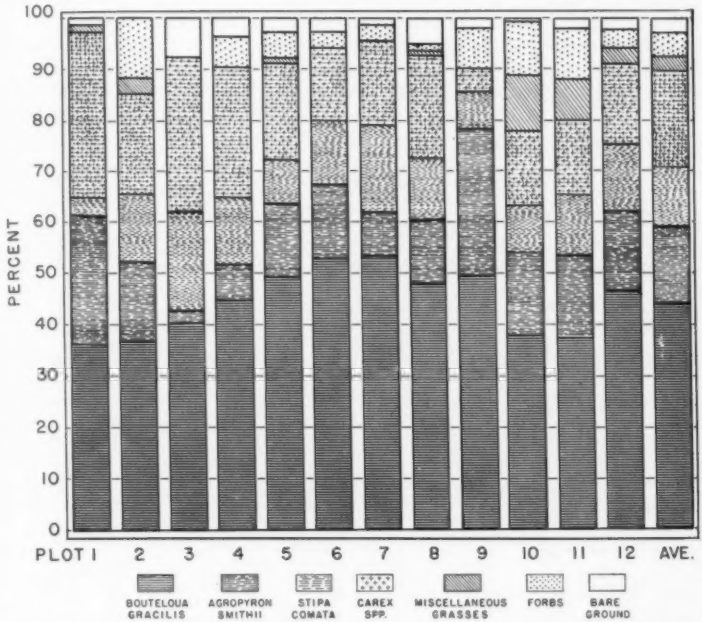

FIGURE 6.—Analysis by the point method showing percentages of cover each species contributes to the total area of plant cover and percentages of bare ground in each of the 12 plots (on the basis of 200 points per plot).

Agropyron contributed only 15 percent and *Stipa* 12. This method appears to overemphasize the importance of *Bouteloua* and *Carex* as compared to *Agropyron*, because of the greater height growth and weight of the last species.

FREQUENCY-ABUNDANCE METHOD

Thirty quadrats each 1 m² were distributed an equal number of paces apart along two lines, running lengthwise, and well separated on the experimental area of 5 acres. For each quadrat an estimate was made of the abundance of each species according to the scale: S=1 to 4 stalks or small plants per quadrat; I=5 to 14; F=15 to 29; A=30 to 99; and VA=100 and over. The number of species found

on each square meter varied from 7 to 15 and averaged 10.6. The total number of species in all quadrats was 35. The summarized data are presented in table 11. Frequency is expressed in percentage, calculated by dividing the number of quadrats in which the species was found by the total number of quadrats (30) that were examined. *Koeleria*, for example, was found in only 1 quadrat, so its frequency is 1 divided by 30, or 3 percent. Average abundance was calculated for each species by adding the abundance values and dividing by the number of quadrats in which the species were present. For example, *Aster* is present in 4 quadrats with abundance estimates as follows: *S*, *I*, *F*, *A*. Adding the numerical values assigned to each of these letters according to the scale $S=1$, $I=5$, $F=15$, $A=30$, and $VA=100$, gives the sum of 51. This sum is then divided by the number of quadrats in which *Aster* appeared (*4*), which gives the average abundance, 12.7. A valuable index number of the abundance of each species on the area as a whole was secured by multiplying the frequency by the average abundance. The maximum possible range for any species present is from 100 to 0.03. It is usually impossible for any species except the chief dominants to reach figures above 50 or 60. The higher the index value, the more important is the species in the community.

TABLE 11.—*Frequency and abundance of species on the experimental area as determined by listing and estimating abundance on 30 square-meter quadrats, July 20, 1933*

Species	Frequency	Average abundance	Frequency × abundance	Species	Frequency	Average abundance	Frequency × abundance
	Percent				*Percent*		
Agropyron smithii	100	62.7	62.70	*Artemisia frigida*	37	6.2	2.29
Bouteloua gracilis	100	100.0	100.00	*Draba nemorosa*	3	1.0	.03
Stipa comata	100	45.0	45.00	*Phlox hoodii*	17	7.4	1.26
Calamagrostis montanensis	17	4.2	.71	*Vicia sparsifolia*	7	5.0	.35
Koeleria cristata	3	5.0	.15	*Aster multiflorus*	13	6.5	.84
Carex filifolia	70	96.7	67.69	*Petalostemon purpureus*	3	1.0	.03
Carex stenophylla	87	32.4	28.19	*Sideranthus spinulosus*	7	3.0	.21
Malvastrum coccineum	37	7.1	2.63	*Opuntia fragilis*	3	1.0	.03
Plantago purshii	97	21.8	21.15	*Lappula occidentalis*	3	1.0	.03
Lepidium densiflorum	40	2.7	1.08	*Leptilon canadense*	3	1.0	.03
Linum rigidum	43	1.3	.56	*Carduus undulatus*	3	1.0	.03
Hedeoma hispida	87	11.5	10.00	*Mamillaria vivipara*	3	1.0	.03
Lygodesmia juncea	60	3.6	2.16	*Opuntia polyacantha*	3	15.0	.45
Chenopodium leptophyllum	3	1.0	.03	*Psoralea argophylla*	10	8.3	.83
Androsace occidentalis	23	13.0	2.99	*Antennaria aprica*	3	15.0	.45
Lithospermum linearifolium	17	1.8	.31	*Astragalus crassicarpus*	3	5.0	.15
Gaura coccinea	7	3.0	.21	*Allium reticulatum*	3	1.0	.03
				Astragalus missouriensis	3	1.0	.03
				Total			352.66

The method shows that while *Agropyron smithii*, *Bouteloua gracilis*, and *Stipa comata* were equally well distributed over the area, each having a frequency of 100 percent, *Bouteloua* was far more important because of its greater abundance (value of 100 compared to 62.7 for *Agropyron* and 45 for *Stipa*). By this method, then, the relative importance of species may be rapidly determined.

A comparison was made of four different sizes of sample areas in determining frequency abundance. Their measurements in decimeters were 2.5 by 4 (0.1 m^2), 2.5 by 8 (0.2 m^2), 5 by 8 (0.4 m^2), and 10 by 10 (1 m^2). They were located on a piece of native grassland as

nearly homogeneous as could be found. The total extent was about 5 acres. A sufficient number of sample areas were taken to make the total area equal 30 square meters. Of the 0.1-m² size 300 samples were taken; of the 0.2-m² size, 150; of the 0.4-m² size, 75; and of the 1-m² size, 30. Comparisons were also made of only 30 sample areas of the four different sizes. The data are summarized in table 12.

TABLE 12.—*Frequency of species according to different numbers and sizes of sample areas, Camels Hump range, 4 miles northeast of Sentinel Butte, July 12, 1932*

Species	30 samples, 0.1 m²	30 samples, 0.2 m²	30 samples, 0.4 m²	30 samples, 1.0 m²	300 samples, 0.1 m²	150 samples, 0.2 m²	75 samples, 0.4 m²
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Agropyron smithii	100	100	100	100	100	100	100
Bouteloua gracilis	100	100	100	100	99	100	100
Carex stenophylla	73	80	83	83	73	77	81
Carex filifolia	40	40	50	57	29	35	37
Salsola pestifer	37	47	63	83	33	43	64
Malvastrum coccineum	23	27	40	47	27	35	44
Stipa comata	27	33	40	43	24	33	40
Euphorbia glyptosperma	10	30	43	67	14	32	39
Phlox hoodii	7	13	13	17	3	6	8
Linum rigidum	3	7	7	20	2	7	11
Hedeoma hispida	3	7	7	17	5	9	8
Schedonnardus paniculatus	3	3	3	3	1	1	1
Linum lewisii	3	3	3	3	1	1	1
Plantago purshii	0	3	17	33	6	13	19
Stipa viridula	0	7	10	13	3	6	13
Opuntia polyacantha	0	3	7	13	1	3	13
Koeleria cristata	0	3	7	7	0	1	3
Collomia linearis	0	3	3	10	2	3	3
Helianthus petiolaris	0	0	3	7	1	2	4
Psoralea argophylla	0	0	3	7	1	1	3
Lepidium densiflorum	0	0	3	7	2	3	7
Eurotia lanata	0	0	3	3	1	1	3
Lactuca pulchella	0	0	3	3	0	0	1
Draba nemorosa	0	3	3	3	0	1	1
Musineon divaricatum	0	0	3	3	1	0	0
Achillea lanulosa	0	0	0	3	0	0	0
Ratibida columnaris	0	0	0	3	0	0	0
Lappula occidentalis	0	0	0	3	0	0	0
Androsace occidentalis	0	0	0	3	0	0	0
Artemisia frigida	0	0	0	3	1	3	7
Gaura coccinea	0	0	0	0	1	1	3
Astragalus caespitosus	0	0	0	0	1	1	1
Artemisia gnaphalodes	0	0	0	0	1	0	0
Potentilla pulcherrima	0	0	0	0	1	0	0
Lygodesmia juncea	0	0	0	0	1	0	0
Chenopodium leptophyllum	0	0	3	3	0	0	1
Allium reticulatum	0	0	0	0	0	0	1
Lithospermum linearifolium	0	0	0	0	0	0	1
Cheirinia aspera	0	0	0	0	0	1	1
Mamillaria vivipara	0	0	0	0	0	0	1
Grindelia squarrosa	0	0	0	0	0	1	0
Total number of species	13	19	26	31	28	28	32

An analysis of the data indicates that for the type of vegetation under study (*Bouteloua-Agropyron-Carex-Stipa*) there are some important differences in the results when the same total area is used. The total number of species included in each set of sample areas varied from 28 in the two smallest sizes to 32 in the 0.4-m² size. The percentage of frequency was, however, progressively lower for many of the less important species as the size of sample area decreased. This is to be expected, for as the number of sample areas becomes much larger, the infrequent species do not show a correspondingly increased number of appearances in them. A comparison with the listing and point methods indicates that the use of a large number of small sample areas is better than a small number of large sample

areas because the latter overemphasizes the less important species such as forbs.

When only 30 sample areas are used the number of species decreases as the size of sample area decreases. The number of species decreases less rapidly than the size of the sample area. There were 31 species represented in the thirty 1-m^2 sample areas, 26 in the 0.4-m^2 size, 19 in the 0.2-m^2 size, and 13 in the 0.1-m^2 size.

A detailed comparison of the summarized field data (table 12) leads to the conclusion that for this type of vegetation at least thirty and preferably about fifty, 0.4-m^2 (5 by 8 dm) sample areas will give best results. The reasons for this conclusion are that 30 sample areas of this size yield for the more important species results that are similar to those secured by the square-meter size and that the less important species are not given as much prominence. By using about 50 samples the secondary species would be reduced in importance even further, which would make the results more comparable to those secured by other methods. A new scale of abundance should be tested for this size of sample area so that frequency-abundance index figures may be calculated.

The 0.4-m^2 size (5 by 8 dm) of sample area has another advantage in that it is much more convenient to handle in the field than the unwieldy square-meter size. It is further desirable because the field counting can be done more rapidly and accurately than on the square-meter size.

COMPARISON OF METHODS

The chart quadrat presents a map or chart giving the location of each plant and areas of clumps, mats, etc. It is a very useful method for tracing the history of individual plants, development of erosion gulleys, or other disturbances, and their effects upon the plants, as well as for many other purposes. It is, however, a time-consuming method even when the pantograph is used.

The area-list and count-list methods are more rapid than the chart-list method. They are especially valuable in studying permanent quadrats. All three methods may be used in various combinations. For example, in a permanent quadrat where the specific object is to determine whether or not a certain species, such as *Calamovilfa longifolia*, is increasing in area, the exact location of each stalk of this grass may be charted and the other plants occurring in the quadrat only listed. The amount of detail that is necessary depends on the object of the particular quadrat. Because of the limitations of time and money, comparatively few permanent quadrats can be charted in one season. For many problems it is necessary to use a number of sample areas instead of a few. The use of area-list and count-list methods are then of considerable advantage.

The frequency-abundance and point methods are much more rapid than the chart or the various listing methods. Neither of them, however, yields data that are as satisfactory for statistical analysis as the listing methods do. The use of listing methods, followed by statistical analyses of the data, are more quantitative and lead to the formulation of conclusions that are more definite and reliable than those obtained by the use of the frequency-abundance and point methods. Both of these methods, however, furnish valuable additional data, even on the same experimental areas where the listing

and charting methods have been used, chiefly because they can be used to cover a large area in a short time. It is hardly valid to compare the results obtained by the different methods with one another, but the data secured by one method may be compared with profit to those secured by the same method on other areas or on the same area in different years. These two methods yield data that give a more complete analysis and record of the vegetation that is under study, and since native vegetation is usually highly heterogeneous it is desirable to have as complete a picture of it as possible.

The list-weight, frequency, and point methods were compared by arranging species in five groups, summarizing the data given in preceding tables, and calculating percentages. These data are presented in table 13. The total area included in the list-weight sample areas was 9.6 m^2. For the frequency-abundance method 30 m^2 were used. The greatest differences in the three methods were found in the percentages assigned to *Agropyron* and to the group comprising *Bouteloua* and *Carex* spp. *Agropyron* was high and *Bouteloua-Carex* low in the list-weight method because of the obviously greater thickness and weight of the leaves and stems of the former. For a given weight of herbage *Bouteloua* and *Carex* have a much larger surface of leaf and stem than *Agropyron*. This accounts for the large differences between the list-weight and point methods. By the former method *Bouteloua-Carex* contributed 49.9 percent and *Agropyron* 28.3 percent of the total weight. By the point method *Bouteloua-Carex* made up 65.3 percent and *Agropyron* only 15 percent of the vegetative cover. Such differences may be expected since by one method weight and by the other method area are measured. The percentages secured by the frequency-abundance method were usually intermediate. Those for *Bouteloua-Carex* and for forbs were nearer the list-weight figures, those for *Agropyron* and *Stipa* were nearer the percentages of the point method.

TABLE 13.—*Comparison of methods showing the percentage of vegetation or pasturage contributed by each group on plateau, 1933*

Groups	List-weight method, 96 sample areas		Frequency-abundance method, 30 quadrats		Point method, 2,400 points
	Grams	*Percent*	*F×A, index number*	*Percent*	*Percent*
Bouteloua gracilis and *Carex* spp.	568.2	49.9	195.8	55.4	65.3
Agropyron smithii	322.6	28.3	62.7	17.8	15.0
Stipa comata	110.9	9.7	45.0	12.7	12.0
Miscellaneous grasses	28.3	2.5	.9	.2	2.3
Forbs	109.9	9.6	48.9	13.9	5.4
Total	1,139.9	100.0	353.3	100.0	100.0

Comparisons of these methods were made on three other areas in the vicinity of Sentinel Butte. All of these were on the upland type of vegetation, similar to that on the experimental plateau on which most of the work presented in this paper was done. One of these additional areas had been overgrazed by cattle. The summarized data are presented in table 14. The sizes of sample areas and quadrats were the same as those used above.

TABLE 14.—*Comparison of methods on the same range for different years and on different ranges, showing the percentage of vegetation of pasturage contributed by each group*

Group	List-weight method		Frequency-abundance method		Point method
	Grams	*Percent*	*F×A index number*	*Percent*	*Percent*
HIGH-QUALITY RANGE, 4 MILES EAST OF SENTINEL BUTTE, 1932 [1]					
Bouteloua gracilis and *Carex* spp.	116.0	66.5	154.4	65.3	70.0
Agropyron smithii	53.7	30.8	67.7	28.6	23.6
Stipa comata	1.8	1.0	5.8	2.5	3.5
Miscellaneous grasses			.6	.3	.4
Forbs	2.9	1.7	7.9	3.3	2.5
Total	174.4	100.0	236.4	100.0	100.0
HIGH-QUALITY RANGE, 4 MILES SOUTHEAST OF SENTINEL BUTTE, 1932 [2]					
Bouteloua gracilis and *Carex* spp.	30.7	69.8	143.5	56.4	61.1
Agropyron smithii	10.0	22.7	54.0	21.2	17.4
Stipa comata			17.4	6.8	8.7
Miscellaneous grasses	.8	1.8	15.3	6.0	4.9
Forbs	2.5	5.7	24.5	9.6	7.9
Total	44.0	100.0	254.7	100.0	100.0
OVERGRAZED PASTURE, 14 MILES SOUTH OF SENTINEL BUTTE, 1932 [3]					
Bouteloua gracilis			100.0	55.7	72.2
Carex spp.			15.5	8.6	6.3
Agropyron smithii			13.6	7.6	9.3
Stipa comata			4.9	2.7	3.5
Miscellaneous grasses			2.2	1.2	.9
Artemisia frigida			8.7	4.8	1.7
Malvastrum coccineum			5.7	3.2	.9
Miscellaneous forbs			29.1	16.2	5.2
Total			179.7	100.0	100.0

[1] 10 sample areas, 30 quadrats, 1,000 points.
[2] 4 sample areas, 30 quadrats, 300 points.
[3] 30 quadrats, 300 points.

The high degree of similarity in the percentages secured by the three methods for *Bouteloua-Carex* on the first area in table 14 is surprising. On the second area the percentages are much farther apart. For *Agropyron* higher percentages are secured by the list-weight method in both of the first two areas than by the other two methods. The percentages secured by the frequency-abundance method in most cases are nearer the list-weight figures than are the point-method figures. It is evident that on the second area four sample areas were insufficient for the list-weight method because *Stipa comata* did not occur in them while it made up 6.8 and 8.7 percent of the vegetation by the other two methods.

In the case of the overgrazed pasture, where there was an abundance of unpalatable forbs, it is significant that the point method does not demonstrate this as well as the frequency-abundance method. The reason for this is that the forbs usually have thicker and heavier leaves and stems in relation to their area than such species as grama grass and sedges. Because of the greater area exposed by the two latter they are hit more often by the pins used in the point method than are the more compact forbs.

In some investigations of grasslands, especially of the reconnaissance type, the use of both the frequency-abundance and point methods are adequate. An example of this is the study of the relationship between vegetative cover and kind of soil in a region. This problem requires examination of vegetation and soils on many uniform areas. In some erosion problems where it is important to study the relation of vegetative cover to bare soil the point method should be valuable in conjunction with chart quadrats. In investigations where listing and charting methods are desirable to use, but time and assistance are so limited that it is impossible to use them, much valuable data may be secured by employing the two rapid methods.

For determining as quantitatively as possible the effects of different grazing practices upon the vegetation, a few well-located permanent quadrats for studying specific problems should be supplemented by a sufficient number of sample areas located at random. For the type of vegetation studied in the present investigation the conclusion appears to be warranted that it is desirable to locate the sample areas in plots instead of scattering them over the experimental area without reference to plots. Analysis by the variance method showed that the minimum size of plot to use in this type of vegetation is 2 by 3 rods with twenty-four 0.1-m^2 sample areas per plot. When this size of plot or the 2 by 4 rod size, with thirty-two 0.1-m^2 sample areas in each, is used, 7 replications for each kind of treatment are more satisfactory than 5. The data secured from these sample areas arranged in plots, by means of the area-list, count-list, or weight-list methods, lend themselves very nicely to Fisher's analysis of variance method (*1*). For other types of vegetation, such as the short-grass plains, true prairie, bunch-grass prairie, etc., further investigation is essential before recommendations can be made regarding number, size, and shape of plots or sample areas.

SUMMARY

This investigation was conducted in the vicinity of Sentinel Butte in western North Dakota in "mixed prairie" vegetation consisting of the dominants *Bouteloua gracilis*, *Agropyron smithii*, *Carex filifolia*, *C. stenophylla*, and *Stipa comata*.

Data secured by the area-list, count-list, and weight-list methods for the following sizes and shapes of sample areas were compared by various statistical procedures: 2.5 by 4 dm, 2.5 by 8 dm, 5 by 4 dm, and 5 by 8 dm.

The sample areas were arranged in 12 plots on an experimental area of about 5 acres. Each plot was subdivided into units of 1 square rod. In each quarter square rod was located at random 1 of the 5- by 8-dm sample areas, which was subdivided into four 2.5- by 4-dm sample areas. In this way a total of 32 of the largest size sample areas and 128 of the smallest size sample areas were listed in each of the 12 plots. Data from different sizes and shapes of plots, varying from 1 by 1 to 2 by 4 rods, were analyzed. Different numbers of plots and sample areas were also compared.

It was found that of the different numbers and kinds tested the most desirable number, size, and shape of sample areas to use were thirty-two 0.1 m^2 (2.5 by 4 dm) per plot of 8 square rods (2 by 4 rods) or 24 per plot of 6 square rods (2 by 3 rods), so arranged

that there was one sample area located at random in each quarter square rod.

It was found that of the different sizes and shapes of plots tested the minimum desirable size to use was one measuring 2 by 3 rods containing 24 sample areas. A comparison of different numbers of the 2- by 3-rod and 2- by 4-rod plots, each containing 24 or 32 sample areas, showed that a minimum of seven replications was necessary in order to secure reliable results.

The use of sample areas located in plots is recommended in place of sample areas not in plots.

Analyses of the vegetation by the point and the frequency-abundance methods are presented and the results compared with each other and with those obtained by other methods.

The area-list, count-list, and weight-list methods, sometimes in combination with charting methods, are essential in numerous kinds of intensive investigations. Data and analyses presented in this paper appear to warrant the conclusion that quantitative and reliable results may be secured when listing is done on sample areas arranged in plots, both in sufficient numbers for statistical treatment. Valuable supplementary information will be furnished by the use of the point and frequency-abundance methods since large areas can be quickly covered by these methods.

For extensive investigations, and where time and assistance are limited, the use of the point and frequency-abundance methods, supplemented by permanent quadrats, is recommended.

These conclusions cannot be applied to other types of grassland, as the short-grass plains, true prairie, and bunch-grass prairie without further investigation.

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COTTON CROP LOSSES FROM PHYMATOTRICHUM ROOT ROT [1]

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INTRODUCTION

The root-rot disease caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar has been reported on 771 cultivated and noncultivated host plants (*12*)[3]. Although root rot reduces the cotton crop of the United States probably to a greater extent than any other cotton disease, the losses occasioned by it have been difficult to estimate, and the estimates made by different agencies have varied widely. The difficulty of estimating losses from *Phymatotrichum* root rot appears to be due to two causes. The first and obvious one is that cotton plants killed by root rot may nevertheless bear a partial crop. The second is the close relation that exists between the effect of rainfall on the prevalence of root rot and on the growth of the cotton plant. In the Southwestern States, where root rot occurs, lack of soil moisture is ordinarily the most important factor limiting growth of both the plant and the fungus parasite. In dry years, root rot causes little damage; yet the yield of cotton is usually low. In years of relatively high rainfall, root rot is more prevalent (*9*); nevertheless the yield of cotton is generally higher. Thus the effect of root rot on yields may be masked by the additional direct effect of the weather on the cotton plant. A simple comparison of cotton yields with the occurrence of root rot over a period of years is therefore not sufficient to give an idea of the loss that may correctly be attributed to the disease. This paper presents an analysis of field-plot data which has furnished a tentative basis for estimating losses from root rot, and it also presents estimates of root-rot losses in Texas in 1928 as an example of actual estimation on this basis.

LOSSES FROM PLANTS SUCCUMBING AT DIFFERENT TIMES DURING THE SAME SEASON

It is well known that cotton plants killed by root rot may bear at least a partial crop, and that in general the earlier the plants succumb to the disease, the greater the loss of cotton (*15*). A quantitative study of the effect of earliness of attack on yields has been made with data on individual plant yields. These were obtained in 1931 in a number of rows of Startex (Texas Station no. 7000) cotton plants growing in the check plots of an experimental field, on Lufkin fine sandy loam soil, at College Station, Tex. This field had been in grass prior to 1929, when it was plowed, limed at the rate of 2 tons per acre, which adjusted the reaction of the soil to about pH 8.0, and planted thereafter to cotton. The check plots had been given uniform cultural

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[3] Reference is made by number (italic) to Literature Cited, p. 857.

treatment. Root rot had been introduced into this field by artificial inoculation (*10*) during the summer of 1929. By 1931 the disease was thoroughly established, and in many plots killed almost every plant by the end of the season. The plants of the 1931 crop were grown at intervals of 3 to 4 inches in the rows, and cultivated in the usual way. In addition, the plots were irrigated thoroughly at intervals during the season when necessary to insure conditions favorable for spread of root rot. Soil-moisture conditions in this field were therefore not representative of normal dry-farming field conditions in 1931, but, instead, of unusual soil-moisture conditions that would allow uninterrupted spread of root rot during the season.

At weekly intervals during the season, plants that had been killed by root rot and those that had wilted from advanced injury by the

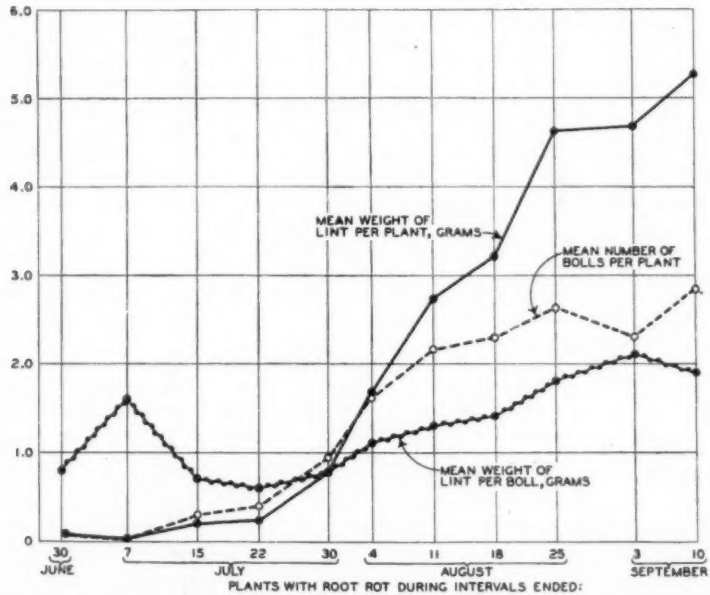

FIGURE 1.—Mean weight of cotton lint per plant and per boll, and mean number of bolls per plant, picked from cotton plants succumbing to root rot during different periods in the summer of 1931. On those plants that were still normal on September 10, the mean number of bolls was 2.3; the mean weight of lint per boll was 2.0 g; and the mean weight of lint per plant was 4.6 g.

disease were marked with small stakes bearing letters to indicate the dates when they had succumbed. Plants growing in the exterior, guard rows of the plots were omitted from the study, but every plant in the selected inside rows was used. Cotton from these plants was picked on September 10 and again on September 28. The cotton from each plant was placed in a separate envelope, allowed to dry in the laboratory for 1 month, and weighed. The seed cotton from each plant was ginned separately on a small electric gin, and the lint and seed weighed separately for each plant.

The yields obtained are summarized in table 1, and the mean values for number of bolls per plant, weight of lint per boll, and weight of lint per plant, are shown graphically in figure 1. These results confirm

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TABLE 1.—*Yield of cotton on plants that succumbed to Phymatotrichum root rot at different periods during the summer of 1931*

[In experimental plots at College Station; cotton was picked Sept. 10 and 28]

Periods during which plants succumbed to root rot	Plants	Bolls picked		Yield of seed cotton		Yield of cottonseed			Yield of lint cotton			
		Total	Mean per plant	Total weight	Mean weight per plant	Total weight	Mean weight per boll	Mean weight per plant	Total weight	Mean weight per boll	Mean weight per plant	Percentage lost per plant, on basis of normal plants
	Number	*Number*	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
To June 30	77	[1] 8	0.10	16.6	0.22	10.0	1.25	0.13	6.6	0.8	0.09±0.03	98
July 1 to 7	49	[2] 1	.02	3.8	.08	2.2	2.20	.05	1.6	1.6	.03± .02	99
July 8 to 15	33	10	.30	16.9	.51	9.3	.93	.28	6.7	.7	.20± .06	96
July 16 to 22	45	18	.40	27.7	.62	16.1	.89	.36	10.6	.6	.24± .06	95
July 23 to 30	55	51	.94	114.6	2.08	69.6	1.36	1.27	43.2	.8	.79± .14	83
July 31 to Aug. 4	46	74	1.61	204.4	4.45	124.9	1.69	2.71	77.7	1.1	1.69± .21	63
Aug. 5 to 11	93	201	2.16	666.7	7.18	396.4	1.97	4.26	255.2	1.3	2.74± .31	41
Aug. 12 to 18	42	96	2.29	345.4	8.22	203.5	2.12	4.85	134.9	1.4	3.21± .38	30
Aug. 19 to 25	29	76	2.62	337.7	11.65	200.4	2.64	6.91	134.2	1.8	4.63± .54	----------
Aug. 26 to Sept. 3	121	277	2.30	1,436.4	11.88	843.6	3.05	6.97	567.2	2.1	4.69± .33	----------
Sept. 4 to 10	56	159	2.84	760.3	13.60	449.0	2.82	8.02	294.4	1.9	5.26± .47	----------
All plants with root rot	646	971	1.50	3,930.5	6.08	2,325.0	2.39	3.60	1,532.3	1.6	2.37± .11	48.5
Normal plants	235	547	2.33	2,782.7	11.87	1,659.3	3.03	7.06	1,082.3	2.0	4.61± .26	0
All plants	881	1,518	1.72	6,713.2	7.62	3,984.3	2.62	4.52	2,614.6	1.7	2.97± .11	35.6

[1] 2 of these 8 bolls were on plants that recovered and were still green on Sept. 10.
[2] This boll borne on a plant that recovered and was green on Sept. 10.

the common observation that early death of cotton plants from root rot causes much greater reduction in yield than later death. Plants that succumbed to the disease 2 months or more before the first picking bore only an insignificant crop of cotton, and plants that succumbed only 5 weeks before harvest bore not more than half as much cotton as the normal plants. The loss was due partly to decreased weights of lint per boll and partly to reduced numbers of bolls on the diseased plants. The 646 plants with root rot averaged only 2.37 g of cotton per plant, as compared with 4.61 g of lint on the plants that were still free of root rot on September 10.

The plants that succumbed during the 3 weeks immediately preceding harvest, between August 19 and September 10, averaged no loss but instead gave slightly higher yields than the normal plants. The differences here were not large but agreed with the results of some earlier unpublished studies by the writers. In these earlier studies with data from the Temple substation, a steady increase was found in the number of bolls per plant, beginning with 0.6 boll per plant on those that showed signs of root rot prior to June 19, and running up to about 4.5 bolls on plants that remained normal to the end of the season. However, the plants that succumbed to root rot after the last week in August produced a still higher average of about 5 bolls per plant. Two probably supplementary explanations of increased yields on plants that succumb to root rot very late in the season may be offered. (1) As root-rot spots enlarge during the season, these plants in the peripheral areas probably benefit from increased moisture and nutrient supplies made available as a result of lack of competition from the adjacent plants already killed by the disease. (2) More initial infections and also greater spread probably occur in the naturally moister parts of the field rather than in the naturally drier parts. Higher average yields would then be expected on surviving plants in the vicinity of root-rot spots because of the more favorable moisture conditions. The increased growth prior to infection is apparently more than sufficient to counterbalance the ill effects of late attack of root rot.

The total of 881 plants multiplied by the 4.61 g of lint averaged by the normal plants, gives 4,061.4 g of cotton as the "expected" yield in the absence of root rot.[4] The actual total was only 2,614.6, leaving an estimated loss of 1,446.8 g, or 35.6 percent of the expected yield. The ratio [5] of this percentage reduction in yield (35.6 percent) to the percentage of plants that succumbed to root rot (73.3 percent) was 0.485.

Additional studies on the quality of the lint and seed obtained from these plants are presented in other papers (8, 13).

YIELDS IN FIELD PLOTS AS RELATED TO PREVALENCE OF ROOT ROT

Another approach to the problem of estimating losses has been made by using field-plot data. Records of yields of cotton in two duplicate permanent cotton plots, each one-tenth of an acre in size,

[4] The term "expected yield" is used in this paper, in the customary way, as meaning the actual yield plus the estimated loss from the disease. The loss in terms of percentage, or the percentage reduction in yield, is obtained by dividing the loss by the expected yield rather than by the actual yield.

[5] Similar ratios of percentage reductions in yield to percentages of plants that succumbed to root rot have been designated throughout this paper as "loss-estimation ratios." These ratios are factors by which percentages of plants succumbing to the disease may be multiplied to furnish estimated percentage reductions in yields.

at the former site of Texas Agricultural Experiment Station substation no. 5, near Temple, are given in table 2.[6] Cotton was grown continuously in these plots from the time the station was established in 1912, until 1927, and percentages of plants killed by root rot were recorded separately for the plots, usually in October but sometimes in November, beginning with 1916. Plot F14 was located in an area of Lewisville clay, and plot C33 was mostly in Bell clay but extended partly into the Lewisville clay. The plots were located at precisely the same distance from the nearby woods in which boll weevils may have hibernated, and dusting and similar treatments were always the same in the two plots. The plots were planted from 1916 through 1919 to Mebane Triumph cotton (Texas Station no. 725) and from 1920 through 1927 to Belton (Texas Station no. 5984), a variety which in comparative tests at this substation did not yield quite so well as the Mebane Triumph. Fortunately, these strains were compared in repeated tests at this same substation (*6*); so it was possible to adjust the yields recorded for 1916 through 1919 to figures representative of yields that might have been expected in these plots had the Belton cotton been used in these earlier years also. These adjusted yields, as given in table 2, were used in analyzing these data.

TABLE 2.—*Rainfall, cotton yields, and prevalence of Phymatotrichum root rot in permanent cotton plots at substation no. 5, Temple, Tex.*

Year	Rainfall [1]					Permanent cotton plot C33		Permanent cotton plot F14	
	May	June	July	August	May–August	Adjusted yields of lint per acre [2]	Plants killed by root rot	Adjusted yields of lint per acre [2]	Plants killed by root rot
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Pounds*	*Percent*	*Pounds*	*Percent*
1916	6.92	1.85	1.31	0.81	10.89	176.6	28.5	174.0	32.0
1917	3.19	1.18	2.67	1.19	8.23	93.3	14.5	107.5	17.4
1918	1.19	1.57	-------	.07	2.83	30.5	8.5	36.4	5.3
1919	3.20	7.87	.93	5.10	17.10	176.6	45.0	100.9	60.0
1920	4.80	3.06	3.66	10.41	21.93	234.0	37.5	255.4	55.0
1921	1.42	5.42	2.14	.87	9.85	239.3	3.0	59.2	78.0
1922	6.70	.43	.79	1.55	9.47	121.8	1.4	114.0	26.7
1923	1.23	2.97	1.80	.75	6.75	131.7	29.5	90.6	70.3
1924	5.38	1.32	.28	.61	7.59	122.8	44.4	78.9	73.9
1925	2.60	.31	.51	1.89	5.31	29.6	42.0	33.7	37.3
1926	2.14	2.65	1.40	1.31	7.50	72.0	75.5	91.0	79.5
1927	3.77	7.02	1.27	.08	12.14	45.0	49.2	35.0	72.1
Mean	-------	-------	-------	-------	9.96	122.8	31.6	98.8	50.6

[1] Rainfall data from Texas Experiment Station Bulletin 399 (*6, table 1*).
[2] Yields recorded 1916–19 were for variety Mebane Triumph (T. S. no. 725) and those in other years for Belton (T. S. no. 5984). The 1916–19 recorded yields have been multiplied by 0.84 to approximate yields of Belton cotton, on the basis of comparative tests detailed in Texas Station Bulletin 399 (*6, table 19*).

MULTIPLE CORRELATION ANALYSIS

The data were first analyzed separately for each plot by the Bean method of graphic multiple correlation (*2, p. 229*). This method consists of repeated approximate fitting of a curve for one variable to the data, followed by fitting of a curve for another variable to the residuals from this first curve. By this method it is possible to determine readily the final net regression lines expressing the individual effect of each of the various independent variables on the dependent variable.

[6] These data were recorded during successive years by Superintendents D. T. Killough, A. B. Cron, H. E. Rea, and Henry Dunlavy.

In the present study, final net regression lines were determined to show the apparent effect of percentage of plants killed by root rot on the yield, after allowing for the effect of the differences in rainfall; and the effect of the rainfall on yield, after allowing for root rot. The analysis was extended to test the possibility that a time trend might

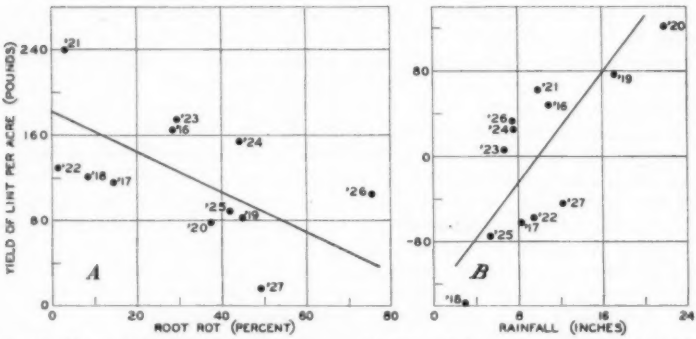

FIGURE 2.—Net regression lines for continuous cotton plot C33: *A*, Relation of percentages of plants killed by root rot to the yields, after allowing for the effect of May–August rainfall on yields; *B*, relation of the May–August rainfall to yields, after allowing for the effect of root rot.

have affected the yields also, as might have been expected from depletion of soil fertility by continued erosion or continued cropping, but no evidence of such a time trend was found.

The graphs for both plots (figs. 2 and 3) show the markedly favorable effect of increased rainfall on yields, and the equally definite

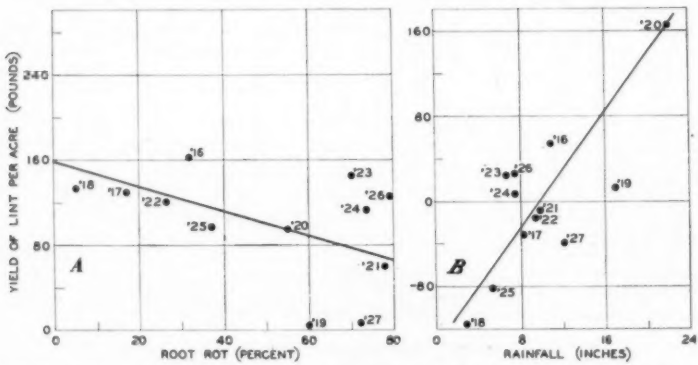

FIGURE 3.—Net regression lines for continuous cotton plot F14: *A*, Relation of percentages of plants killed by root rot to the yields, after allowing for the effect of May–August rainfall on yields; *B*, relation of the May–August rainfall to yields, after allowing for the effect of root rot.

but unfavorable effect of increased root rot on yields. With plot C33, the relation of yields to rainfall was apparently curvilinear, and fitting the data to a curve concave downward yielded a slightly higher multiple correlation than was obtained with the linear regression lines, but the relation of root rot to the corrected yields was not affected. On the other hand, the corrected yields in plot F14 showed no

evidence of a curvilinear relation to rainfall. Linear regression lines were used in the present analysis for both plots.

The standard errors of estimate and indexes of multiple correlation were calculated from the regression lines by the formulas (*2*):

$$\overline{S^2}_{1.23}=\frac{n\sigma_s^2}{n-m}, \text{ and}$$

$$\overline{R^2}_{1.23}=1-\frac{\overline{S^2}_{1.23}}{\sigma_1^2}\left(\frac{n-1}{n}\right).$$

The coefficients of multiple correlation corrected for number of variables, between the yields of cotton and the combined effects of root rot and the rainfall from May through August, were, for plot C33, $R=0.65$; and for plot F14, $R=0.62$. These two factors considered in the analysis therefore apparently accounted for approximately 43 percent of the variation in yield from year to year in plot C33, and for approximately 39 percent of the variations in yield in plot F14. The standard errors of estimate determined for these data were 55 pounds for plot C33 and 49.8 pounds for plot F14.

The average apparent effect of root rot on yields during the period covered is shown by the slope of the net regression lines. For plot C33, with each additional 1 percent of plants killed by root rot there was apparently a loss of about 1.9 pounds of cotton. A total of 133 pounds is indicated as the probable average yield in this plot in the absence of root rot; hence this loss of 1.9 pounds represented a loss of 1.04 percent of the expected yield for each 1 percent of plants killed. In plot F14 each 1 percent of plants killed by root rot was accompanied by an average loss of 1.17 pounds of lint, equivalent to 0.74 percent of the expected yield of 158 pounds.

ANALYSIS BY DIRECT COMPARISON

Obviously, variations in the yields in these plots may have been due in part to seasonal factors other than those taken into account in the multiple correlation analysis. This was evident from the corrected yield figures, which in both plots were well above the estimated figures for 1916, 1923, 1924, and 1926, and below the estimated values for 1919 and 1927. Little additional information was obtained by extension of the multiple correlation analysis to include the rainfall data by individual months or by 2-month periods. However, another approach to the problem has been made on the assumption that in a given season the yields were probably affected by general seasonal variables operating over extensive areas, such as weather conditions and insect infestations, to approximately the same proportionate extent in both plots. The yields in plot F14 for each year were divided by the corresponding yields in plot C33. In the absence of root rot or other local variables affecting the yields, the resulting ratios, F14/C33, should have been fairly similar in different years. Instead these ratios (given in table 3) varied from 25 percent in 1921 to 126 percent in 1926, and, as shown in figure 4, these differences in yield ratios were strongly associated with differences in prevalence of root rot in the two plots. The correlation between these values was determined mathematically as $\bar{r}=-0.83$, and the slope of the regression

line, $Y=111.02-1.13\ X$. This equation is converted to apply to yields in the individual plots by calculations such as shown in table 30.

Assuming no root rot in either plot, $X=0$ and $Y=111.02$ percent. Y also equals yield of F14 divided by yield of C33. With a yield of 100 pounds in plot C33, the yield in plot F14 would be 111.02 pounds. Now, assuming 10 percent root rot in plot F14 but none in plot C33, $X=10-0=10$ percent; and $Y=111.02-11.3=99.72$ percent. With the yield in C33 assumed still as 100 pounds, the yield in F14=99.72 percent×100 pounds=99.72 pounds. This represents a loss of 11.3 pounds from the expected yield in the absence of root rot, which would still be 111.02 pounds. The reduction in yield is, then, 11.3/111.02, or 10.2 percent. This percentage, divided by the percentage of root rot (10), yields a final loss-estimation ratio, 1.02, which represents the average relation between the percentage reductions in yield and the percentages of root rot. This ratio is intermediate between the ratios determined separately for the two plots in the multiple correlation analysis.

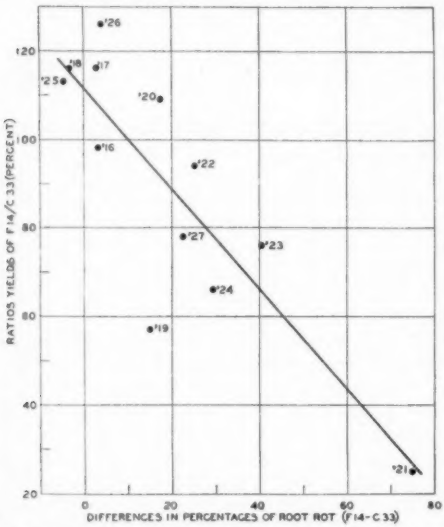

FIGURE 4.—Correlation of percentages of plants with root rot, using the differences in the percentages in plots F14 and C33 (1916 to 1927), and the yields of cotton, using ratios of the yields in the two plots; $r=-0.83$.

TABLE 3.—*Analysis of effect of root rot on cotton yields by direct comparison of duplicate permanent cotton plots F14 and C33*

Year	Ratios of yields, F14/C33	Differences in root-rot percentages, F14–C33	Estimated losses from root rot		Estimated yields in the absence of root rot	
			Plot F14	Plot C33	Plot F14	Plot C33
	Percent	*Percent*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1916	98	3.5	84	72	238	249
1917	116	2.9	23	16	131	109
1918	116	−3.2	2	3	38	34
1919	57	15.0	159	149	260	326
1920	100	17.5	326	145	581	379
1921	25	75.0	231	7	290	246
1922	94	25.3	43	2	157	124
1923	76	40.8	252	57	352	189
1924	66	29.5	241	101	320	224
1925	113	−4.7	20	22	54	52
1926	126	4.0	390	241	481	313
1927	78	22.9	98	45	133	90
Mean	89.5	19.04	155.8	71.7	254.6	194.6

This loss-estimation ratio has been applied to the actual data to find the estimated losses from root rot and the estimated yields in the absence of root rot. These values (given in table 3) were calculated by the formula,

$$\text{Estimated yield} = \frac{\text{Recorded yield}}{100 - (1.02 \times \text{percentage plants with root rot})}$$

The mean losses from root rot, over the 12-year period, are estimated at about 72 pounds per acre for plot C33, and about 156 pounds per acre for plot F14, or, respectively, 37 percent and 61 percent of the yields to be expected in the absence of root rot.

EFFECT OF DISTRIBUTION OF RAINFALL ON THE INFLUENCE OF ROOT ROT ON YIELDS

The loss-estimation ratios derived above are obviously to be considered approximate averages, rather than precise representatives of the relations between root rot and loss in every year, even for the period covered by these data. In figure 4, only 2 of the 12 dots for the individual years fell very close to the regression line representing the average relation for the period. However, considering the rainfall during these years (table 2) it becomes evident that the divergence of the other 10 years from the average relation was not accidental but instead was clearly correlated with the distribution of rainfall during the summer months.

In the 5 years above the regression line in figure 4 (1917, 1920, 1922, 1923, and 1926) there was in general unusually uniform rainfall during the summer. Fairly even distribution continued throughout the season in 4 of the 5 years of this group. On the other hand, there was a decisively different distribution of rainfall in the 5 years (1916, 1919, 1924, 1925, and 1927) that fell below the regression line in figure 4. Characteristically, exceptionally heavy rainfall (6 inches or more) in May or June was followed by several months of relatively low rainfall with less than 1 inch of rain in one or more of these months. An apparent discrepancy in these groupings occurred in 1922. But in 1922 seven-tenths of the heavy May rainfall fell in the first 4 days, and nine-tenths of the rainfall in the first half of the month, apparently too early to exert the same effect on spread of root rot and on yields as resulted in 1916 and 1924, when six-tenths to eight-tenths of the May rainfall fell in the second half of the month.

The relation brought out here between distribution of summer rainfall and crop losses occasioned by a given percentage of root rot cannot be considered as very significant statistically. Nevertheless, it is in exact accord with the results discussed in the first section of this paper, on the yield from cotton plants which succumb to root rot at various times prior to the date of picking, and also with the conclusions of Taubenhaus and Dana (*9*) on the relation of continued rainfall during the season, at the Temple substation, to continued spread of root rot. Heavy early rainfall followed by drought caused unusually high crop losses for a given percentage of plants killed by root rot, presumably because unusually high proportions of the plants with root rot succumbed early in the season. Plants killed prior to August would have borne practically no crop. On the other hand, during years in which rainfall continued during the summer, the

crop losses were unusually low for a given percentage of root rot, presumably because in such seasons root rot continued to spread during the summer, and many of the plants succumbed to root rot after the crop was at least partly mature, increasing the recorded percentages of root rot without contributing proportionately to the actual losses.

COMPARISON OF COLLEGE STATION AND TEMPLE DATA

The discussion above has shown that for the two permanent cotton plots at Temple, an average loss-estimation ratio of about 1.02 apparently agreed nearly perfectly with the recorded yields and the recorded percentages of root rot. This ratio was double the ratio of 0.485 derived from the single year's results at College Station under artificial irrigation. Continued rainfall during the summer has been shown to lower the loss-estimation ratio for that season. A still lower ratio appears entirely logical for cotton grown under irrigation.

The effect of natural local differences in yield was probably a more important factor under field conditions at Temple than it was at College Station. It is obvious from figures 2 and 3 that soil moisture is a very definite limiting factor in the growth of the cotton plant in this region. In almost all fields there are likely to be considerable local differences in soil conditions, including the moisture supply, within relatively short distances. Plants growing in the areas of moister soil probably bear more cotton than plants growing in naturally drier areas. In the absence of root rot, two-thirds of the crop of a given field might be borne on one-half of the plants growing in the moister areas. But local prevalence of root rot is also favored by the additional soil moisture, and the moisture supply is the limiting factor also in the spread of root rot. Therefore, the disease is more likely to occur and to spread more rapidly each year on the plants in the naturally relatively moister parts of the field. With 50 percent root rot, most of the plants in the moister areas of the field might be involved. If these plants normally bore two-thirds of the crop, the total yield for the field would then be reduced to 50 percent of a normal crop even though these diseased plants averaged half the yield of the remaining normal plants. Relatively high ratios between percentage reductions in yields and percentage of plants with root rot can therefore be considered a reasonable expectation, and such was actually the case for the plots at Temple.

ESTIMATION OF COTTON CROP LOSSES FROM ROOT ROT

METHODS

Suggestions for future estimation of losses from root rot cannot be otherwise than arbitrary and tentative, since the information available at the present time is limited to the Temple and College Station data analyzed. From these data, two possible methods may be suggested.

(1) The data required for the first method are percentages of cotton plants killed by root rot at approximately 7 weeks and again at approximately 3 weeks before the date of first picking. Subtract the first percentage from the second, to find the percentage of plants that succumbed during 3 to 7 weeks before harvest, and divide this

difference by 2. Add to the quotient the entire percentage of plants killed 7 weeks before harvest. The sum is the estimated total percentage reduction in yield.

This method is based on the results at College Station which showed that the earlier in the season a given percentage of cotton plants succumb to root rot, the greater is the crop loss. Plants killed 7 weeks or more prior to the first picking bore practically no crop, plants killed 3 to 7 weeks before harvest bore approximately half the crop borne by normal plants, while plants that succumbed during the 3 weeks just before picking bore practically a normal crop. These results are of course strictly applicable only to the particular conditions under which they were obtained, and using them for future estimation of losses may introduce errors of an extent indeterminate without further data of this nature.

(2) The second method requires only a single count of the percentage of plants with root rot at time of first picking. If necessary, an estimate of the percentage of plants with root rot is used instead. Multiply this percentage by a "loss-estimation ratio" to obtain directly the estimated percentage reduction in yield.

The loss-estimation ratio tentatively suggested for use in Texas is 0.9. This ratio is between the ratios determined separately for two permanent cotton plots at the Temple substation in a study of 12 years' records of yields, root rot, and rainfall; and is somewhat below the ratio derived in a simultaneous study of the paired data for the two plots. It is to be noted that this loss-estimation ratio supersedes the ratio suggested earlier (*3, 4*) after study of only a portion of these data. This ratio of 0.9 will probably be more accurate for seasons in which wet and dry months alternate, approaching the customary if not the mean rainfall for most areas in which *Phymatotrichum* root rot occurs, and will be somewhat biased when the seasonal distribution of rainfall departs greatly from this distribution. For seasons with May or June rainfall of 6 inches or more followed by a long period of definitely deficient rainfall, the loss-estimation ratio of 0.9 is probably somewhat too low, and a ratio of 0.95 may be used instead; while for seasons with rainfall continuing fairly uninterruptedly during the summer, the ratio is probably slightly too high, and a ratio of 0.85 may be substituted.

LOSSES IN TEXAS IN 1928

As an example of the estimation of crop losses from root rot, the data available for 1928 have been utilized in the estimations summarized in table 4. These data were from several sources. From October 1 to 15, one of the writers (Taubenhaus) made an extensive survey through the major cotton-growing areas of Texas for the purpose of estimating the severity of root rot. This survey included 41 counties and estimation of root rot in 1,750 fields. Additional information about the relative severity of root rot on October 1 was kindly made available by H. H. Schutz, formerly statistician of the Bureau of Agricultural Economics, United States Department of Agriculture. His data included reports on the percentages of plants with root rot, sent in by crop reporters from 150 counties. Both sets of data were most extensive for the areas and the counties in which cotton was most widely grown, and estimates of root rot from

the two sources agreed fairly well for these areas. For counties not covered specifically from either source, estimates were made arbitrarily by the writers. These estimates were based on the writers' general knowledge of the distribution and prevalence of root rot and on the rainfall for 1928, as well as on the severity of the disease in adjacent counties from which reports were available.

For convenience in estimating the losses, the State was divided into 11 large sections according to the prevalence of root rot in 1928. These sections coincided in the eastern part of the State with the natural geographic and soil divisions of the State, as delimited by

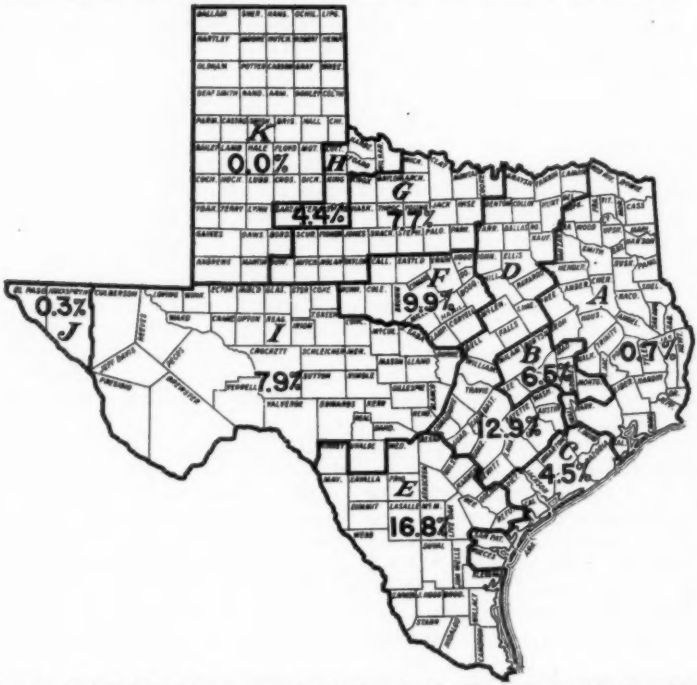

FIGURE 5.—Estimated losses from cotton root rot in Texas in 1928. The figures give the estimated mean percentage reductions in yield for the various sections of the State, lettered as in table 4; also see text.

Carter (*1*), except that the borders were extended to county lines and a few counties placed in adjoining sections to agree with the distribution of root rot. The 10 sections of the State in which root rot occurs are designated *A* to *J* in figure 5.

The sectional average percentages of plants with root rot were obtained by weighting the estimates of root-rot percentages for individual counties by the size of the cotton crop harvested in 1928. The estimated percentage reductions in yield were calculated from the percentages of plants with root rot, using a basic loss-estimation ratio of 0.9 as suggested above. By using these estimated percentages of reduction in yield, the yield in the absence of root rot has been calculated and the estimated loss determined.

TABLE 4.—*Sectional summary of cotton-crop losses from Phymatotrichum root rot in Texas in 1928, averaged from data from several sources as discussed in the text*

Section of the State			1928 cotton crop [1]	Cotton plants with root rot, final weighted mean estimate	Estimated reduction yield [2]	Estimated yield in absence of root rot [3]	Estimated loss from root rot [4]
Designation in map, (fig. 5)	Name of section	Counties					
		Number	*500-pound bales*	*Percent*	*Percent*	*500-pound bales*	*500-pound bales*
A	East Texas	44	869,115	0.8	0.72	875,418	6,303
B	Eastern marginal	8	250,602	7.2	6.48	267,966	17,364
C	Central coast	10	373,431	5.0	4.50	391,027	17,596
D	Blackland Prairies	33	1,822,272	14.3	12.87	2,091,440	269,168
E	Rio Grande Plain	28	280,128	18.7	16.83	336,814	56,686
F	Central	14	255,262	11.0	9.90	283,310	28,048
G	North Texas plains and prairies	23	376,625	8.6	7.74	408,221	31,596
H	Northern marginal	8	129,749	4.9	4.41	135,735	5,986
I	Edwards Plateau and Trans-Pecos	40	127,881	8.8	7.92	138,880	10,999
J	El Paso Valley	2	59,414	.3	.27	59,575	61
K	Northwest Texas plains	44	565,460	0	0	565,460	0
	Entire State	254	5,109,939	8.9	7.993	5,553,846	443,907

[1] Data on yields from Cotton Production in the United States. Crop of 1928 (16).
[2] Calculated from the loss-estimation ratio of 0.9.
[3] Obtained by dividing recorded yields by (100 percent−percentage reductions in yield).
[4] Obtained by subtracting recorded yields from estimated yields in the absence of root rot, or by multiplying estimated yields by the percentage reductions in yield.

The general prevalence and severity of root rot in the various parts of Texas is represented fairly accurately by these estimates for 1928, as given in figure 5. There are three sections of the State in which root rot is of little importance. The disease is not known to occur in the northwest plains section (*K*). In east Texas (*A*) root rot is present only in limited areas. Many of the soils are acid enough to be unsuitable for the disease, which occurs only locally in the rarer neutral to alkaline soil areas (*5*, *14*). In the El Paso Valley (*J*), which includes only El Paso and Hudspeth Counties, the cotton crop is grown under irrigation. The severity of root rot there is limited probably by the accumulation of alkali salts in the soil to a sufficient extent to hold back the root-rot fungus, which is somewhat more susceptible to alkali salts than is the cotton plant. Throughout the remainder of the State, the dark, nonacid upland soils predominate. Root rot is very widespread on such soils, particularly on those which are calcareous, and its destructiveness from year to year is limited to a great extent only by weather conditions.

For the entire State, the 1928 losses are estimated at about 444,000 bales, or just under 8 percent of the estimated yield in the absence of root rot. This estimate for the State is probably conservative for 1928. Furthermore, since nearly one-third of the cotton crop was grown in the three sections in which root rot is relatively unimportant, this weighted State average is much too low to represent the average loss from the disease in the more typical root-rot sections. The weighted loss for sections *B* to *I*, inclusive, averages instead about 11 percent.

A further economic effect of the root-rot disease is the lowering of the quality and consequent sale value of cotton lint, and probably also of cottonseed, from infected plants that bear partial crops. This additional loss from root rot is discussed in detail in other papers (*8*, *13*).

LOCAL VARIATIONS IN ROOT ROT IN INTERPRETATION OF LOSSES

It may not be amiss to point out that from the nature of the disease, the average estimates of losses from root rot for the State, for sections of the State, or for counties, are in fact averages of widely varying percentages. The individual counties within the sections marked off in the map vary somewhat in the relative areas occupied by soils with reactions favorable for the root-rot fungus, and vary correspondingly in occurrence and severity of root rot. Furthermore, even within a fairly homogeneous soil area, the destructiveness of root rot varies from a number of causes. Thus, it becomes increasingly destructive in fields grown continuously to cotton or other susceptible crops. There is also a definite tendency for periodic increase and decrease in the severity of root rot over periods of years, even in fields grown continuously to cotton and quite aside from seasonal weather conditions (*7, 11, 15*). Estimation of losses is therefore a matter of determining the average of losses that may range from a trace, even in areas in which the disease is widely prevalent, up to nearly complete loss.

For example, in Brazos County a large part of the upland Lufkin soils vary from slight alkalinity to slight acidity. Root rot is of sporadic occurrence in these soils and probably causes losses exceeding 5 percent of the cotton crop in only a small proportion of those fields in which it occurs. But the disease is widespread and highly destructive in the many fields of cotton grown in a belt of productive, neutral to definitely alkaline, Crockett and Wilson soils across one end of the county. Losses here may run in individual fields to 50 percent or higher during seasons favorable for the disease. Thus, the mean estimate of 6 percent of cotton plants killed in this county for 1928 represents very clearly an average of widely varying percentages of loss, rather than of a generalized, widespread light infestation.

SUMMARY

Estimation of the reduction of the cotton crop by *Phymatotrichum omnivorum* root rot is difficult because plants killed by the disease may bear a partial crop, and conditions that favor development of root rot also favor the growth of the cotton plant.

In an experimental plot in which the cotton was picked separately from each plant, it was found that the numbers of bolls per plant, the weight of lint per boll as well as per plant, and the weight of seed per boll as well as per plant, were all lower with plants that had succumbed to root rot. Normal plants bore an average of 4.61 g of lint per plant, and the plants with root rot, only 2.37 g. Plants that succumbed 2 months or more before the first picking bore only an insignificant crop; plants that succumbed 5 weeks before harvest bore only half of a normal crop; while there was no observed loss from the death of plants during the 3 weeks immediately preceding harvest.

The coefficient of multiple correlation of yields, May-June-July-August rainfall, and percentages of plants with root rot (using data from continuous cotton plots for 12 consecutive years) was 0.65 for one plot and 0.62 for another. In one plot, the average reduction in yields from root rot was 1.04 times the average percentage of plants killed and in the other plot it was 0.74 times the percentage killed.

The plot data were analyzed again by pairing the results during the various years, and a corrected correlation between ratios of yields in the two plots with the differences in percentages of root rot of $\bar{r}=-0.83$ was obtained. From this analysis, the percentage reductions in yield due to root rot averaged 1.02 times the recorded percentages of plants that succumbed to the disease.

Two tentative methods for the estimation of cotton crop losses from root rot are suggested. By the first method, the estimated percentage reduction in yield is the sum of the percentage of plants killed by root rot 7 weeks prior to picking time plus half of the percentage of plants that succumbed during 3 to 7 weeks before harvest. By the second method, the estimated percentage reduction in yield is the product of the percentage of plants killed by root rot by picking time multiplied by a "loss-estimation ratio." A ratio of 0.9 is tentatively suggested for use in Texas.

The cotton crop losses from root rot in Texas in 1928 were estimated from data obtained in a survey through 41 counties, and crop-reporter estimates from 150 counties. The State was divided into 11 regions according to the prevalence of root rot. Weighted estimates of crop losses in the different regions varied from 0 to 16.8 percent. For the entire State, the mean weighted estimate of cotton plants with root rot was 8.9 percent. The estimated reduction in yield was about 8 percent, equivalent to a loss of about 444,000 bales.

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